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NOTES ON THE GENUS LEMANEA IN NORTH AMERICA

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No adequate knowledge of the structure, development, and relationship of the genus *Lemanea* existed until the appearance of SIRODOT'S classic work in 1872.[2] Prior to this time the knowledge of species rested almost entirely on superficial characters. The few studies of internal structure were so incomplete as to add diagnostic features of very little value. In a number of cases, forms representing distinct sections of the genus were placed in the same species, sometimes even mixed in a single collection, or packet. Since SIRODOT'S work, there is no longer any difficulty in recognizing the sec-

[1] After the publication of the monograph of *Lemanea* in 1890 (ATKINSON, GEO. F., Monograph of the Lemaneaceae of the United States. Ann. Botany **4**: 177–229. 1890), Professor ATKINSON received a number of specimens of this group from several parts of North America. During the two years preceding his death in 1918, he devoted considerable time to a study of this material of *Lemanea*. He also studied most of the available material in North America of which he could learn by correspondence with institutions and collectors whose herbaria he thought probably contained collections of this genus. A number of individuals contributed specimens or permitted the retention of a small portion from the packets for his study. All this material, together with Professor ATKINSON'S notes on *Lemanea*, are deposited in the Cornell University herbarium, in which all the known species of North America are thus represented.

It is to be regretted that Professor ATKINSON'S untimely death prevented the publication, as he had planned, of his notes on the American species of *Lemanea* and their

[*Footnote 1 continued on following page*]

[2] SIRODOT, S., Étude anatomique, organogénique et physiologique sur les Algues d'eau douce de la Famille des Lémanéacées. Ann. Sci. Nat. Bot. Ser. V. **16**: 1–95. Pls. 1–8. 1872.

tion of the genus to which any specimen belongs, provided one takes the little trouble necessary in determining the one feature in the internal structure of the sexual shoots which clearly distinguishes the two sections of the genus.

The species are remarkable for their peculiar habitat, as well as for the season of the year during which they begin and complete their growth. They live only in fresh water, where the water is turbulent, as in swift streams, at waterfalls or rapids, or especially on the downstream side of large stones in rather deep, strong-flowing streams. Rarely do they occur in sluggish river water. The specimens occur on the edge of rock ledges in rapids or on the rock surface at the foot of waterfalls where the water often strikes with great force, or on the eddying side of boulders or rocks.

The species grow only during the cold months of the year. Sometimes in mountain rivulets the winter growth may be retarded, so that maturity may not be reached in the icy water until sometime in the summer. In some regions, where the autumn season is warm, the cold weather for beginning of growth may not arrive until winter is well advanced, so that maturity may not be reached until late spring. But in the streams in the region of Ithaca, New York and in central North and South Carolina, in fact generally throughout

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distribution. These notes, which were practically ready for publication, have been edited with the hope of making available the results of his intimate knowledge and study of this group of interesting algae.

A number of the specimens which Professor ATKINSON studied, especially those in the Cornell University herbarium, are indicated in the text by letters. This does not necessarily mean that the same species or duplicates of a given number may not be represented in another herbarium, nor are all the collections of any one herbarium indicated. The herbaria are indicated as follows:

C, Cornell University
Calif., University of California
F, W.G. Farlow
M, University of Michigan
N.Y., New York Botanical Garden
U.S., United States National Museum
Y, Yale University

While certain changes and omissions have been made, the manuscript remains substantially as prepared by Professor ATKINSON. Yet it would be unjust to hold Professor ATKINSON responsible for any discrepancies that may appear. Despite the effort to retain his style and individuality, the quality of the notes is no doubt altered by passing through the hands of another.—W. C. MUENSCHER.

their range, the species reach maturity during late winter or early spring. They thrive, therefore, in water very near the freezing point, often under ice. From this period on, the sexual shoots, by weathering and whipping in the strong current, become more and more broken, while surface features in the characteristic antheridial zone are worn down. For this reason, specimens gathered during the summer, the usual season for such collections, have frequently lost so many distinctive features that *specific* determination is often rendered extremely uncertain.

The spores germinate in late autumn or early winter, and form a branched filamentous or cellular sole which gives rise to the erect, branched, *Chantransia*-like but sterile protonemata. The sexual shoots arise as branches from the protonemata, either near the base, along the middle, or in the superior parts. The *Chantransia*-like protonema gives rise to rhizoids, usually near the base, but also along the middle region. These rhizoids add to the sole on the surface of the rock. Rhizoids also arise from near the base of the sexual shoots, and contribute also to the sole. The rhizoids also produce *Chantransia*-like protonemata and even sexual shoots. It is interesting to note that the interrelations and functions of the sole, the *Chantransia*-like protonemata, and the sexual shoots parallel remarkably the interrelations and functions of the protonemata and leafy stems (sexual shoots) of the mosses.

The branch which forms the sexual shoot is much stouter, even at its origin, than are the sterile branches. It grows by a single apical cell, from which short, tabular cells are cut off behind by cross walls, in regular succession. The apical cell is short and convex. The tabular cells are at first broader than long. Each tabular cell develops into a definite morphological and structural unit of the sexual shoot. The sexual shoot consists of "nodes" and "internodes." The shoots vary in length from 20 up to 200 or more internodes. The nodes are short, and in most species are more or less prominent because of their greater diameter than that of the internodes. The internodes are comparatively long, three to six times longer than their diameter, are either cylindrical, slightly constricted, or shaped like an hourglass. The sexual shoots, therefore, present a more or less "nodulose" or "torulose" appearance, whence the specific names of *nodosa*, *torulosa*, *catenata*, etc.

Each tabular cell develops into a definite morphological and structural unit. A single node is an antheridial zone, the antheridia being massed in definite fields forming a whorl, or in a ring over its surface. A single internode is a procarp zone, since the procarp branches are distributed throughout the length of the internode, or are limited to the middle area or to the ends; that is, to the portions of the internodes adjoining the nodes. The morphological or structural unit, which is developed from a single tabular cell of the young sexual shoot, consists of a single internode and the adjacent halves of each adjoining node. Description of the successive stages in the development of one of these units of the sexual shoots is too complicated, and would require too much detail, for the purpose of this paper. It is in brief a process of cell division, combined with branching.

The terminal branches remain closely united, forming the three to five or more cell-layered wall of the shoot. The internal, or primary, branches elongate rapidly and undergo no further branching (with the special exceptions in one section of the genus). The result is a hollow sexual shoot with four primary branches arranged cruciately. They stand at the middle of each internode. There is a central axis consisting of a single row of greatly elongated cells, one cell for each "morphological unit" of the shoot. The elongation of this cell is downward into the adjacent morphological unit, reaching nearly to the middle of the latter, where it joins the corresponding cell of that unit. This central axis cell then bears the four primary ray cells near its apex or distal end, but in the middle of its corresponding procarp zone.

In one group of species (*Sacheria*) these ray cells are L- or T-shaped, the arm or arms of the L or T being closely applied to the wall of the sexual shoot. In the other group (*Eulemanea*) the ray cells are straight, and the distal end of each is separated from the wall by an oval or pyriform cell. From the distal ends of the ray cells, in both groups of species, two to four secondary branches arise, some extending upward, some downward. These secondary branches are long and consist of numerous cells placed end to end. In *Sacheria* they lie close to the wall throughout their entire length, while in *Eulemanea* they are separated from it by oval or pyriform cells, at regular intervals, except near the antheridial zone. On their outer

surface these secondary (or in *Eulemanea* tertiary) branches give rise to more numerous short branches, which themselves branch rapidly several times, forming the cellular wall. The inner cells of the wall are larger and fewer in number; the outer ones smaller and more numerous. The outer portion of the wall consists, in some species, of one layer and, in other species, of two layers of small short cells forming a compact palisade. The homologous outer layers of cells, in certain definite areas on the antheridial zone, are the antheridia, each of which contains a short, stout sperm cell, or "spermatium." The antheridia, therefore, form a complex or group of cells, terminating the secondary branches which meet in the antheridial zone from the two adjacent procarp zones. The cortex of the shoot is formed from the tips of the lateral branches arising from the secondary branches.

These secondary branches, which line the inner surface of the wall, give rise also to the procarp branches. Each procarp consists of a single row of four to ten short cells. The terminal one to three cells lie in the wall, while the trichogyne from the carpogone extends to the surface. The secondary branches, which bear the procarps, were termed by SIRODOT "tubes lateraux," by ATKINSON "generative filaments," by OLTMANNS "Längsfäden." After fertilization, the carpogone, or fertilized egg, gives rise to the short ooblastema filaments which bear the branched spore chains, forming the cluster known as the cystocarp. The growth direction of the spore chains is toward the center and in the cavity of the sexual shoot, which becomes more or less filled with carpospores, according to the species or the varying number and size of the cystocarps.

One interesting and important morphological feature, characteristic of certain species, remains to be described. This is the envelopment of the central axis of the sexual shoot by numerous slender threads which arise near the proximal ends of the four primary branches or rays. In all the species investigated, with one exception, these enveloping filaments arise from the under side of the rays, and extend downward in a spiral manner, winding around the central axis, often forming a thick and dense covering. The one known exception is *Lemanea parvula* Sirodot, in France. In this species, according to SIRODOT, the enveloping filaments extend upward.

SIRODOT revised the genus *Lemanea* and recognized two genera. He retained the genus name *Lemanea* for those species in which the enveloping axial filaments from the ray cells are present. For the other group of species, devoid of enveloping axial filaments, and which he considered to be of generic rank, he gave the name *Sacheria* (Ann. Sci. Nat. Bot. Ser. V. **16**:70. 1872). Other characters are correlated with this fundamental one of the presence or absence of filaments enveloping the central axis. Some of them appear to be constant; for example, the forms of the ray cells and the relation of the generative filaments to the wall. Others are variable in certain species; for example, in the *Sacheria* section procarps usually arise in and near the antheridial zone, but in *Lemanea* (*Sacheria*) *fluviatilis* they are distributed throughout the procarp zone also. In the *Eulemanea* section the procarps arise in the middle of the procarp zone, and are usually absent from the region of the antheridial zone. But in *Lemanea pleocarpa* Atkinson they are distributed throughout the procarp zone and occur also in the antheridial zone. In the *Sacheria* section, the antheridial zones are usually more or less warty and the antheridia are confined to these protuberances; while in the *Eulemanea* section the antheridial zone is usually even and the antheridia are usually associated in a broader, narrow, encircling band; but variations occur.

In the *Sacheria* section the procarp branch is short (two to four cells) because the generative filaments lie close to the inner surface of the wall throughout their entire length. In the *Eulemanea* section the procarp branch is longer (five to ten cells) because the generative filaments are separated from the wall by stout cells except in the antheridial region. In the *Sacheria* section the hypogynous cells (those below the egg cell) do not proliferate; they thus remain naked except as they are covered by the group of carpospores. In the *Eulemanea* section many of the hypogynous cells of the procarp proliferate, producing short filaments. This proliferation of the hypogynous cells of the procarp suggests a primitive cystocarp wall. In the *Eulemanea* section proliferation takes place also in the inner wall cells of some species.[3]

[3] In the monograph of the Lemaneaceae of the United States, ATKINSON, while recognizing the great merit of SIRODOT'S work, employed only the genus *Lemanea*, treating *Sacheria* as a subgenus. This procedure was followed by subsequent authors. In the present notes *Sacheria* is treated as a section of *Lemanea*.

SECTION EULEMANEA

LEMANEA ANNULATA Kütz. Phyc. Germ. 261. 1845; Species Alg. 528. 1849; Tab. Phyc. 7:33. pl. 84, fig. 1. 1857.

When the spores are mature, L. *annulata* and *L. fluviatilis* present a close superficial resemblance, and they are apt to be confused unless the internal structure is examined. The difference is then very apparent since they belong to distinct subdivisions of the genus. In *L. annulata* the antheridial zone is light in color, as in the young stages, but the middle of the procarp zone is dark from the mass of spores. *L. fluviatilis*, when the spores are mature, presents alternating light and dark zones, but since the spores are borne next to the antheridial zone, and not in the middle of the procarp zone as in *L. annulata*, the antheridial zone is dark, while the middle of the procarp zone is light, since the violet color fades from the walls at maturity.

California: in rapidly flowing water on the rocky bed of a small stream which becomes dry in the summer, hills about 2 miles southwest of Bloomington, San Bernardino Co., *S. B. Parish*, Mar. 23, 1897, Phyc. Bor. Am. no. 329 (C); in stream Tamalpais, Marion Co., *E. L. Greene*, May, 1886 (Calif.); Corkscrew Falls, near Bluff Lake, San Bernardino Mts., altitude 7400 ft., *S. B. Parish*, June 21–27, 1895 (Calif.); on rocks in swift stream, Mt. Tamalpais, Marion Co., *W. J. V. Osterhout*, July, 1906; in stream, San Luis Obispo, from Herb. *J. W. Bailey* (Calif.); in stream, Santa Margarita, *C. C. Parry*, on Mexican Boundary Survey, Apr., 1850 (N.Y.); in stream, Santa Cruz, *C. L. Anderson*, no date (N. Y.); in stream, Oakland, *C. L. Anderson*, no date (F); streams in vicinity of San Francisco, *T. S. Brandegee* (C); in stream, Univ. Calif., Berkeley, 1871, *Herb., Wolle* as "*L. fluviatilis*" (C); in running water, waterworks of Leland Stanford Jr. University, *N. L. Gardner*, Apr. 19, 1916 (Calif.).

Indiana: Eel River Falls, Owen Co., *L. M. Underwood*, May, 1883, Phyc. Bor. Am. no. 237 (C). This material was formerly determined as *L. catenata* but a re-examination shows that it is *L. annulata*.

Nevada: in stream, Diamond Mts., *Sereno Watson*, U.S. Geol. Exp. 40th Parallel, no. 1546 (Yale).

Oregon: in streams, Oregon City, *A. S. Foster* (F); in streams, eastern Oregon, *W. C. Cusick*, 1886, no. 1353 (F).

Washington: in springs near Rock Creek, Spokane Co., altitude 670 m., *J. H. Sandberg* and *J. B. Leiberg*, June 1, 1893 (U S.); (C).

LEMANEA ANNULATA Kütz. var. **franciscana** Atkinson, var. n.

Sexual shoots slender, 10–20 cm. long, less than 1 mm. in diameter, straight, very pliant, dark violet, blackening when dry, tapering gradually at the base. Procarp zones short, tapering slightly and gradually from the antheridial zone and thus nearly cylindrical. Antheridial band usually regular, but tissue beneath distinctly darker than adjacent wall of procarp zone even at time of anthesis, thus not agreeing with the normal conditions of the species; procarps in middle of procarp zone, the spores even from young stage of the cystocarp giving a darker color to middle portion of procarp zone.[4]

This plant appears to be only a slender and darker-violet variety of *L. annulata*, owing to the habitat of shade and swiftly running water. The darker color of the antheridial band in the young condition violates one of the principal characters of the species. Even in age, when the color fades out greatly from the wall of the sexual shoots, so that a light-colored zone alternates with the middle portion of the procarp zone, darkened by the mass of spores, the antheridial band is still darker in color than the adjacent tissues. But the subadjacent tissue has the same appearance as in normal specimens, forming a slightly depressed ring. This same appearance is sometimes present in large and normal specimens. Even in the older specimens the spores are not mature, since the tips of the chains are slender.

California: in falls in deep, shady ravine, no. 94471 (Calif.).

LEMANEA AUSTRALIS Atkinson. Ann. Botany **4**:218, figs. 19–44, 47. 1890.

The type material of this species is that collected by *Atkinson* in Morgan's Creek, North Carolina. The material was collected

[4] To conform with the International Rules of Plant Nomenclature the description is here Latinized: Stirpes sexuales tenues, 10–20 cm. longae, diametro minores quam 1 mm., rectae, valde flexibiles, atro-violaceae, ad basim sensim angustatae, demum exsiccatae nigrescentes. Procarpiorum zonae breves, paulo sensimque e zona antheridiali attenuatae, quam ob rem fere cylindricae. Vitta antheridialis plerumque regularis, sed caro infra quam murus proximus zonae procarpiorum (etiam ad anthesin) manifeste fuscior, itaque condicionibus normalibus pro specie non congruens; procarpiis ad medio suae zonae positis, sporis (etiam ex cystocarpio juveni) colorem atriorem parti medianae zonae medianalis communicantibus.—EDITOR, BOTANICAL GAZETTE.

throughout the winter, so that all stages of the plant have been observed and carefully studied. The broad antheridial band, the rather prominent antheridial zone, and the constricted procarp zone resemble in a rather striking manner *L. nodosa*, but the *Chantransia* form is very different, as can be seen by comparing SIRODOT's fig. 75 and ATKINSON's figs. 23–26 of *L. australis*.

The material collected at Columbia, South Carolina, clearly belongs to this species. Other material from Maryland, West Virginia, South Carolina, Georgia, and Mississippi is assigned here partly because of the general agreement of the mature sexual shoots, and partly because of the regional distribution. The color of the sexual shoots of this species varies considerably under different conditions. When fresh, before they are very old, the shoots are green or olive-green in color; after standing in quiet water for a time or in the laboratory, however, the violet color often appears and the water in which they are left for some time is stained somewhat violet. Specimens which are dried often undergo slight disintegration changes in which this violet color appears, so that dried material of young shoots, or of old shoots in mass, is often more or less violet in color, together with the dark or blackish color which often appears when the plants dry. In this condition, when moistened in water, individual filaments under the lens often show an olive-green color with a tinge of violet (or in some sexual shoots the violet color is marked), while to the unaided eye there is a distinct violet tinge. Specimens from Rocky Creek near Chester, South Carolina, in the young stage show, strikingly, characters of typical *L. australis*, in which many of the more slender specimens show only a slight undulation, thus resembling *L. grandis;* but the larger and more robust ones have a strong undulating outline, corresponding with the undulating antheridial and procarp zones. These different forms can never be assigned satisfactorily to the proper species until the young stages are collected, including well developed *Chantransia* forms and the sexual shoots at the time of fertilization.

Georgia: on submerged limestone rocks in Burnt Mill Creek, alt. 840 ft., Walker Co., *Percy Wilson*, Aug. 3, 1900 (N.Y.); (C).

Maryland: in stream, Garrett Co., *J. D. Smith*, July, 1878 (U.S.).

Mississippi: on rocks in running water, Meridian, *S. M. Tracy*, June 2, 1897 (N. Y.); (C).

North Carolina: in rapids, Morgan's Creek, Chapel Hill, *G. F. Atkinson*, winter and spring of 1886–1888, Phyc. Bor. Am. no. 38 (C).

South Carolina: on large stones in Broad River, Columbia, *G. F. Atkinson*, March and June, 1889 (C); Stroud's Ford, Rocky Creek, 4 miles east of Chester, *H. A. Green*, Nov. 23, 1895; Tilden's Am. Algae no. 108, May, 1890 (C).

West Virginia: Blackwater Falls, *W. C. Sturgis*, July 17, 1889 (Calif.).

LEMANEA CATENATA Kütz. Phyc. Germ. 261. 1845; Sp. Alg. 528. 1849; Tab. Phyc. **7**:34. (pl. 87. fig. 1.) 1857.

The specimens examined from North America are up to 10 cm. long, olive-green when young and fresh, becoming darker in age, and on drying sometimes of a blackish or blackish-violet color; many shoots, however, still show the olive-green color. Antheridial band narrow, antheridial zone more or less angular, especially when young or medium-grown, but not papillate or tubercular.

The specimens collected at Corkscrew Falls, California, appear to represent typical specimens of this species, although they are small and do not show the strong "*catenate*" character of large examples. They compare well with the smaller authentic specimens in SIRODOT'S *Exsiccata*.

California: Corkscrew Falls, San Bernardino Co., *S. B. Parish*, June 22, 1895, no. 94478 (Calif.); in Kern River, *N. L. Gardner*, Nov., 1916, no. 3507 (Calif.); on rocks, Crane Creek Falls, above El Portal, *W. A. Setchell*, June, 1916, no. 6509 (Calif.).

LEMANEA CATENATA Kütz. forma CAPILLACEA Sirodot. Ann. Sci. Nat. Bot. Ser. V. **16**:80. 1872.

Sexual shoots dark olive-green in age with a faint violet tinge, blackening when dried, slender, straight or slightly curved, not rigid, up to 12 cm. long; tapering gradually at base; antheridial region often somewhat angular and prominent. Antheridial bands not very distant, rather narrow, irregular toward base, often interrupted, tissue beneath the antheridia in age often hypertrophied and then sometimes irregular; procarp zone nearly or quite cylindrical; procarps in middle of procarp zone, spores at maturity often filling procarp zone. In these specimens the spores are not mature and do not darken the sexual shoots. The *Chantransia* stage had disap-

peared, and therefore is not available for comparison with SIRODOT's description.

California: West Canyon, Palm Valley, Riverside Co., Eastern (Desert) base San Jacinto Mt., plants of Southern California, *S. B. Parish*, Apr., 1896, no. 4055 (Calif.); (C).

LEMANEA GRANDIS (Wolle) Atkinson. Ann. Botany 4:219. figs. 46, 50. 1890.

The sexual shoots of type material received from Wolle, when moistened in water are of a sordid olive-green color; nearly straight to more or less strongly curved and measure 4–6 cm. × 0.5–0.8 mm. They are mostly cylindrical but taper toward either end, more gradually so toward the base, rather blunt at the apex, where they have been probably broken and then healed. A few of them show a slight undulation of outline, corresponding in these cases to the slightly broader diameter of the antheridial zone and the slightly but gradually constricted procarp zone.

WOLLE says that "the specimens were growing on stones in rather sluggish water in the river at Bethlehem, Pa." Probably in deeper water where there is a stronger current the sexual shoots would be somewhat stouter.

Specimens collected in a stream at Falkland, Delaware, by *A. Commons*, in 1886, have been assigned to this species. The sexual shoots are somewhat stouter, rather strongly curved, and the greater freedom of the shoots from extraneous matter seems to indicate that they were growing in deeper water where the current was probably stronger than in the case of the specimens collected by *Wolle* at Bethlehem. The sexual shoots of these specimens, moistened in water, show under the hand lens a dark green color. To the unaided eye, or in mass, they are darker in color, with a suggestion of a violet tinge. Some younger specimens, when moistened in water, show this violet tinge to the unaided eye, although under the lens the color is more a dark, dull olive-green. These shoots are mostly cylindrical, but a large proportion show slight undulation in consequence of the alternation of the broad antheridial zone with the slightly constricted procarp zone.

Delaware: in river, Falkland, *A. Commons*, 1886 (F); (C). Pennsylvania: on stones in shallow, sluggish river water, Bethlehem,

Frances Wolle, 1876–1878, Rabenhorst's Alg. Europ. no. 2538 (N.Y.).

Lemanea mexicana Kütz. Tab. Phyc. **7**:34. pl. 86, fig. 2. 1857.

Sexual shoots in tufts, simple or sometimes branched, the branching probably abnormal, resulting from injury or hypertrophy, with regeneration of several to numerous sexual shoots in the region of injury; at and soon after fertilization olive in color, olive-brown when dry, specimens examined stout, rather strongly curved, 4–6 cm. long by 1–1.25 mm. in diameter; wall rather thick, firm and subcartilaginous, at time of fertilization and soon after cylindrical or nearly so, antheridial region only slightly stouter, procarp region cylindrical or very slightly and gradually constricted; antheridial band broad and regular, of a slightly darker tint than the wall of the shoot; often presenting several blunt papillae, or sometimes with papillae very numerous on antheridial zone and elsewhere, probably due to some epiphyte; procarps in the middle of the procarp zone; spores in this material few, young.

Mexico: in stream, Orizaba, *Botten*, Herb. Sullivant.

These specimens were made available for examination through the kindness of the late Dr. W. G. FARLOW who also wrote concerning them as follows: "There is no date on *Botten's* specimen. *Botten* was sent by the London Hort. Society to Mexico in 1850 to collect. Somewhat later he settled at Orizaba where he collected independently. He did not die until 1885, but the specimens of *Lemanea* came from Herb. Sullivant and, as S. died in 1873, the chances are that the *Lemanea* was collected between 1850–70." It is possible that KÜTZING's species was based on material collected by *Botten* for the London Hort. Soc. The appearance of the sexual shoot shown in KÜTZING's fig. C[1] resembles the specimens from Orizaba in the warty character, some of these warts very probably being due to an epiphyte.

Lemanea pleocarpa Atkinson, sp. n.

Sexual shoots in dense tufts, rather stout, 8–20 cm. long, purplish brown when dry, when soaked in water showing a slight purplish tinge but the color is pale, often with portions olive-green, rather abruptly tapering at base, which is more or less curved, antheridial zones not prominent, antheridial band narrow and interrupted below, over the middle and terminal portions broader and more regu-

lar; procarp zone only slightly tapering from antheridial zone except near base in very stout plants where it is more strongly constricted, procarps numerous extending throughout the procarp zone even into the antheridial zone. Carpospores large but rather young, many of them quite young in the specimens examined, *Chantransia* form not seen.[5]

In the more general distribution of the procarps this species resembles *Lemanea fluviatilis* but differs in the subgeneric characters. Although in both species there is a wide distribution of the procarps, in this species they are more abundant in the middle of the procarp zone, while in *L. fluviatilis* they are more abundant in the antheridial zone.

The specimens from Kentucky are younger and more vigorous plants. When pressed upon paper the "torulose" appearance is more striking and the antheridial zones show more prominently. The specimens were remarkably well preserved. In the collections from both localities the plants have a purplish-brown tinge when dry, the older ones from Beaver Creek blackish, although the purplish-brown tinge when soaked in water shows also in the younger plants from Kentucky, under the microscope. There are many places on the filaments which also show a greenish color. Whether the plants are entirely or partly green when fresh is not known.

This is a very interesting species. It is the only one in this section of the genus, which has come under observation, in which procarps and cystocarps are present so near to, and in, the antheridial zone. It is interesting also from a historical point of view, the specimens collected by *Dr. Peter* (1834) and *Dr. Short* (1842) being the earliest collections for North America. The specimens from Kentucky collected by *Dr. C. W. Short* are cited as the type of *Lemanea pleocarpa*, with those collected by *Dr. Peter* as a cotype. The latter specimens,

[5] In accordance with the requirements of the International Rules of Plant Nomenclature, the description is here Latinized: Stirpes sexuales dense torulosae, subrobustae, 8–20 cm. longae, exsiccatae purpureo-brunneae, maceratae vix subpurpureae sed pallidae, saepe nonnullis partibus olivaceo-virides, subabrupte attenuatae ad basim plus minusve arcuatam; zonis antheridialibus non valde perspicuis, vitta antheridiali angusta atque inferne interrupte . . . . ; zona procarpiorum e zona antheridiali tantum paululo attenuata (nisi prope basim pro plantis robustissimis valdius constricta), procarpiis numerosis per omnes partes suae zonae et etiam in zonam anteridialem sparsis. Carposporae magnae sed subjuvenes (pro speciminibus visis multae juvenissimae); *Chantransia* forma non visa.—EDITOR, BOTANICAL GAZETTE.

which were examined, were from the herbarium of Judge DOUGLAS HOUGHTON and are now in the herbarium of the University of Michigan.

HARVEY (Nereis Bor. Am. 67. 1858) reports specimens collected by *Short* in rivers and streams of Kentucky as *L. torulosa*, and makes the interesting comment that the "Globose masses of fructification are attached to the inner surface of the tubular frond, either at the nodes or between them without apparent order."

Kentucky: on rocks in brook near Lexington, *Robert Peter*, March, 1834 (M); on rocks and stones, in rivers and small streams, *C. W. Short*, March, 1842 (C).

Virginia: in Little Falls, Beaver Creek, near Marion, *Anna M. Vail* and *Elizabeth G. Britton*, June 21 and 26, 1892 (C).

LEMANEA TORULOSA Sirodot. Ann. Sci. Nat. Bot. Ser. V. **16**:82. pl. 1, fig. 7; pl. 6, figs. 41, 42; pl. 8, fig. 77. 1872.

In this species the sexual shoots are nearly even throughout, the procarp zones being only slightly and gradually, if at all, narrowed, especially at maturity. When fresh the color is olive with a greenish tinge. All the specimens referred here are small. No material of the *Chantransia* form was examined, but the material from a mountain rivulet, Pa. (Herb. Wolle) appears to be typical so far as can be judged from mature sexual shoots alone. That from Wissahickon Creek is badly parasitized by a species of *Onchybursa*. The collections from Virginia, formerly referred doubtfully to *L. nodosa* Kütz. (Atkinson, Ann. Botany **4**:218, 1890), should be referred here pending a study of good material of both stages.

Pennsylvania: on stones in mountain rivulet, at Narrows, *F. Wolle*, July 1, 1874 (appearing to be good *L. torulosa* although small; Herb. Wolle); on rocks in Wissahickon Creek, Oct., 1854 (F); (C).

Virginia: on rocks in rapids of James River, Richmond, *G. F. Atkinson*, June, 1888 (C); in rapids of small stream near Fredericksburg, *G. F. Atkinson*, June, 1888 (C).

SECTION SACHERIA

Lemanea (*Sacheria*) *fluviatilis* (Linn.)Ag. (as *Lemania*), Dispos. Algar. Sv. [Sv.] Vet.-Acad. Handl. 1814: 40; 45. pl. 2, fig. 2. 1814; Spec. Alg. **2**:4. 1824–1828.

Oregon: in brook (F). These are typical specimens but collector and date unknown.

BAILEY (Am. Jour. Sci. Ser. II. 3:185. 1846) reports *L. fluviatilis* Ag. from Cascade, West Point; Mountain Run, Culpepper Co., and falls in the Rappahannock River above Fredericksburg, Va. The collection from the last named locality proved to be *Tuomeya fluviatilis* Harv. The specimens from the two other localities given by BAILEY do not appear to be preserved in his collection, and it is not possible to say what species they represent; but they probably do not belong to *L. fluviatilis* Ag.

Lemanea (*Sacheria*) *fucina* Bory. Ann. Mus. Nat. Hist. **12**:185. pl. 23, fig. 3. 1808.

This is a highly variable species in the form and branching of the sexual shoots, as previously pointed out (ATKINSON, 1890: pp. 224, 225). The variations relate to the form of the procarp zone, the prominence of the antheridial nodules, and the branching. Four of these forms were recognized as species by SIRODOT, but in this country the forms so grade one into another that it seems preferable to treat them merely as varieties.

Var. β. *mamillosa* Atkinson. Ann. Botany **4**:225. figs. 1–5, 8, 9, 11–18, 55, 56, 58. 1890.

North Carolina: Bolan's Creek and Morgan's Creek, near Chapel Hill, *Geo. F. Atkinson*, winter and spring, 1886–1888. Phyc. Bor. Am. no. 37 (C).

Var. γ. *subtilis* Atkinson. Ann. Botany **4**:225. fig. 54. 1890. *Sacheria rigida* Sirodot. Dame and Collins, Flora of Middlesex County, Mass. 153. 1888.

Massachusetts: on rocks in very rapid water, The Cascade, Middlesex Falls, Melrose, *F. S. Collins*, May 4, 1879 and April-June, 1883; Apr. 14, 1889 and April 15, 1894, Phyc. Bor. Am. no. 36*a* and *b* (C); in stream, Arnold Arboretum, Forest Hills, *B. M. Davis*, May 3, 1895; in streams, Malden, *F. S. Collins*.

Var. δ. *rigida* Atkinson. Ann. Botany **4**:225. figs. 6, 7, 10. 1890. *Lemanea borealis* Atkinson, Torreya **4**:26. 1904.

This is the most common variety of the species and is widely distributed in North America. There is considerable variation in the sexual shoots, especially in size. Much of the material collected in the summer or early autumn is badly water-worn. The most important variation is shown in material from Oregon, which varies toward *L. fluviatilis* in the presence of procarps and spore groups

near the middle of the procarp zone, but not so evenly and regularly distributed, and lacks the color of the sexual shoots of typical *L. fluviatilis*. Material from Nova Scotia, especially that from Pirates Cove, and from Newfoundland (Bay of Islands) presents similar variations. That from Bay of Islands, N. F., was made the type of a new species. Some material from this section, however, cannot be separated from the more typical *rigida*, and an examination of SIRODOT'S *Exsiccata* reveals one collection of his *Sacheria rigida* in which the procarps and spore groups, here and there, approach the middle of the procarp zone. The irregularity of this distribution in SIRODOT'S and our material appears to be due to some unfavorable condition of development rather than to genetic differentiation. It is possible that when material from these widely separated localities can be studied carefully in different stages of development, specifically distinct genetic forms may be found; but for the present it does not seem reasonable to attempt a differentiation.

California: in stream at Little Bear Valley, San Bernardino Mts., *S. B. Parish*, Aug., 1884 (F); (C); on rocks in waterfall, Sacramento River, Limo, Shasta Co., *M. A. Howe*, Aug. 10, 1894 (N.Y.); (C).

Colorado: in cold swift rivers, alt. 7000–9000 ft., near Hot Sulphur Springs, Grand Co., *E. Bethel*. No date given, but the envelope containing plants bears the stamp, "Dec. 7, 1911."

Connecticut: Island Brook Bridgeport, *Isaac Holden*, various collections June to Dec., 1888, 1889; *W. A. Setchell*, 1887 (Calif.); (C); Trading Cove Brook, Norwich, *W. A. Setchell*, June 29, 1890 (Calif.); Stony Brook, Montville, *W. A. Setchell*, June 30, 1890 (Calif.); (C); Mill River, Bridgeport, *Isaac Holden*, July 5, 1891; Great Falls, Housatonic River, *Isaac Holden*, June 27, 1891, Phyc. Bor. Am. no. 34*c* (C); in Ironworks Brook, North Guilford, *D. C. Eaton*, May, 1887 (Y); (C); on stones in swift-running water, Sargents River, Woodbridge, *O. D. Allen*, June 29, 1880 (C); on stones in Hammonasset River, Killingworth, *F. W. Hall*, July, 1874 (F); (C); in stream, Franklin, *W. A. Setchell*, July 2, 1888 (Calif.); (C); Westrock Cascade, New Haven, *D. C. Eaton*.

Maine: on rocks in swift water, Sunk Haze Stream, Greenfield, *E. D. Merrill*, Sept., 1897; on ledge in bed of large brook, Camden, *Alice L. Crockett*, July 10, 1904 (N. Y.); (C); on stones in little

brook near Round Pond, *F. S. Collins*, July 14, 1901 (C); outlet of Echo Lake, Mt. Desert Island, *Isaac Holden*, Aug. 11, 1889.

Maryland: in stream, Garrett Co., *J. D. Smith*, July, 1878 (U.S.).

Massachusetts: Bussey Brook, Jamaica Plain, *E. Faxon*, May 3, 1883 (F); (C); waterfall, Waverly, *A. B. Seymour*, 1913 (F); on rocks in bed of rapid stream, Chester, *Dr. E. Emmons* (N.Y.); in rapids of stream below mill dam, Milltown south of Boston, *G. F. Atkinson*, July, 1888 (C); on mill dam in swift current, Arlington, *W. A. Setchell*, June 11, 1890, Dec. 21, 1890, Phyc. Bor. Am. no. 34*a*, *b* (C).

Minnesota: Lester River, Duluth, *C. Bullard*, Aug., 1902 (F).

Mississippi: on rocks in running water, Meridian, *S. M. Tracy*, June 2, 1897 (N.Y.).

New Hampshire: in Mill Brook, Shelbourne, *W. G. Farlow*, Aug., 1882 (F); (N.Y.); (C).

New York: rapids in Cascadilla Gorge, Cornell Univ. campus, *G. F. Atkinson*, winter and spring, 1885 (C); Triphammer Falls and rapids in Fall Creek, Cornell University campus, Ithaca, N.Y., *W. R. Dudley*, June, 1885, *G. F. Atkinson*, July, 1886; on rocks Taughannock Falls, *W. R. Dudley*, Aug. 1, 1885 (C); lower Taughannock Falls, *W. C. Muenscher*, Apr., 1921[6] (C); on trap rock in brook by mill, Johnson Pond Road, Adirondack Mts., *E. G.* and *N. L. Britton*, Sept. 6, 1900 (N.Y.); (C); on rocks, Twin Glen, Ithaca, *W. C. Muenscher*, May 18 and Dec. 7, 1928 (C); Remington Brook, Ithaca, *W. C. Muenscher*, Dec., 1928 (C); on rocks in rapids of St. Regis River, Brasher Falls, *W. C. Muenscher*, Sept. 6, 1930 (C); in rapids below chasm, Chateaugay River, *W. C. Muenscher*, Sept., 1930 (C); Point Rock Creek, Adirondack Mts., *R. Laubengayer*, July, 1928 (C); on stones in rapids of the Bronx River near the Lorillard Mansion, Bronx Park, N.Y. City, *F. T. McLean*, May 9, 1902 (N.Y.); (C); Bashbish Falls, *R. S. Williams*, Aug. 5, 1900 (N. Y.); (C); on rocks, Conglomerate Ravine, Ellenville, *E. G. Knight* and *N. L. Britton*, Aug. 25, 1883 (N.Y.); (C); on stones in swift water, Pond Brook 2 miles east of Fort Ann, Washington Co., *S. H. Burnham*, June 3, 1914 (C).

[6] A few specimens not seen by ATKINSON, those added to the herbarium of Cornell University since 1918, have been included in these notes on distribution.

North Carolina: Oates Falls, *R. Thaxter*, 1896.

Ohio: in stream, Painesville, *Dr. Beardsley* (N.Y.); (C).

Oregon: on stones in creek near Hood's Ranch, National Forest, *F. V. Coville*, June 25, 1907 (U.S.); on rocks in Power's Creek, Silverton, *A. S. Foster*, no. 1228 (N.Y.); in stream, Forest Grove, *A. R. Sweetser*, May, 1903 (F); Oregon, *Elihu Hall*, 1871 (F).

Pennsylvania: Buttermilk Falls, July 4, 1898 (N.Y.); Stony Creek, *F. Wolle*, July, 1875 (C); in rapid water, Bark Hill, *F. Wolle*, 1874, 1877; in bed of brook, Saw Kill Falls, Pike Co., *G. V. Nash*, July 20, 1909 (N.Y.); (C).

Vermont: Otter Creek Falls, Vergennes, *C. P. Mott*, June 29, 1894; Little Otter Creek, Ferrisburg, *E. Faxon*, July 22 and 28, 1884 (F); (C).

Virginia: in rapids, Potomac River, Harper's Ferry, *E. S. Burgess*, June 5, 1888 (C).

West Virginia: Blackwater Falls, Cheat River, *J. D. Smith*, July 5, 1878 (U.S.).

Canada: on rocks in river at Bellville, *J. Macoun* (N.Y.); (C); on rocks in stream, Owen Sound, Ontario, *J. Macoun*, no. 262.

New Brunswick: in Nepsiquit River, *J. B. Fowler* (F).

Newfoundland: on rocks in waterfall, Bay of Islands, *C. D. Howe* and *W. F. Lang*, Aug. 9 and 10, 1901 (N.Y.).

Nova Scotia: in stream, Pirates Cove, *J. Macoun* (F).

British Columbia: in little waterfall, head of Departure Bay, Vancouver Island, *Mary J. Macoun;* not typical.

Var. ε. *viviana* Atkinson, Ann. Botany **4**:226. fig. 53. 1890. (*Sacheria rigida* var. *viviana* Sirodot Ann. Sci. Nat. Bot. Ser. V. **16**:73. fig. 87. 1872.)

Connecticut: Island Brook, Bridgeport, *Isaac Holden*, Dec., 1888 to June, 1889, Phyc. Bor. Am. no. 35 (Calif.); (C); Stillman's Brook, Bridgeport, *Isaac Holden*, May, 1889 (C).

Massachusetts: Bussey Brook, Jamaica Plains, *E. Faxon*, May 9, 1883 (F); (C); Cascades, Waverly, *B. M. Davis*, May 11, 1894.

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THE SUSPENSOR OF SCIADOPITYS

JOHN T. BUCHHOLZ

(WITH NINETEEN FIGURES)

A peculiar suspensor which has seemed unique and anomalous was described for *Sciadopitys* by ARNOLDI (1). This observation has not been confirmed, and our present knowledge of the embryogeny remains fragmentary. The suspensor is composed of two sections which have a group of small cells between the elongated portions. ARNOLDI described also a group of proliferating cells above the suspensor, and likened this feature to a protocorm. Doubtless the object which he observed was a group of rosette cells or embryos. LAWSON (17), who observed some of the early stages of the embryo, did not describe the rosette, nor did his observations extend into the slightly later stages, which include the formation of the interrupted suspensor figured by ARNOLDI. LAWSON concludes: "on the whole the embryo of *Sciadopitys* is rather unique; it does not bear a close resemblance to either Abietineae, Cupressineae, or Taxaceae." COULTER and CHAMBERLAIN (10) regarded *Sciadopitys* as one of the Taxodineae. The writer has observed a very similar suspensor in *Biota* (6), one of the Cupressineae often erroneously included with *Thuja*, and this discovery has stimulated the present investigation into the embryogeny of *Sciadopitys*. The general situation in *Sciadopitys* was inferred and given in a previous publication (4), in an attempt to harmonize the facts as stated and illustrated by ARNOLDI and LAWSON, but this summary was not then based upon an independent personal investigation.

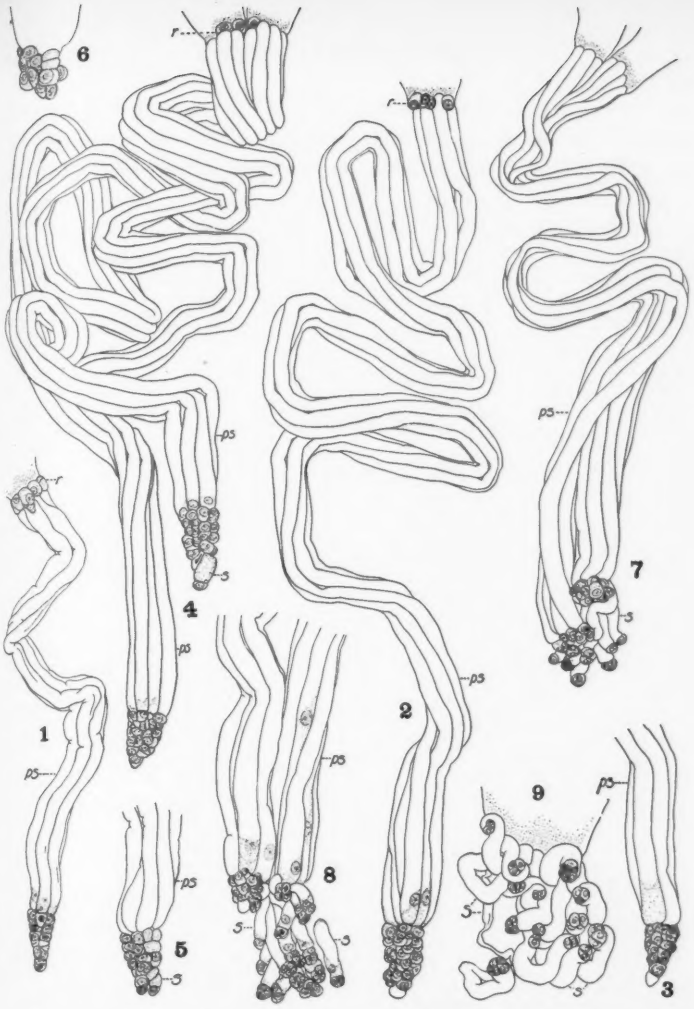
Material and methods

The material used in this investigation was obtained from three sources. Through the kindness of the Director of the Kew Botanical Gardens, Dr. A. W. HILL, arrangements were made for a number of collections of ovules which were killed there in formalin-alcohol at weekly intervals during the summer of 1926. A few cones of *Sciadopitys* were collected also from one of the trees growing on the grounds

of the Country Life Press, Garden City, New York, in the summer of 1926, but the embryos of these were found to be too far advanced to include the early embryogeny; they furnished the examples figured in the oldest stages represented. In the summer of 1927, a single cone was obtained through Kelsey's Nursery from plantings in the region around Salem, Massachusetts. This cone was large and well developed, and contained many embryos in the stages shown in most of the early figures described here. The earliest stages (figs. 1–5) were dissected out on July 12 and 14, and some later stages (figs. 7–9) on July 17, from the ovules of a part of the same cone, which was kept packed in damp moss in the laboratory.

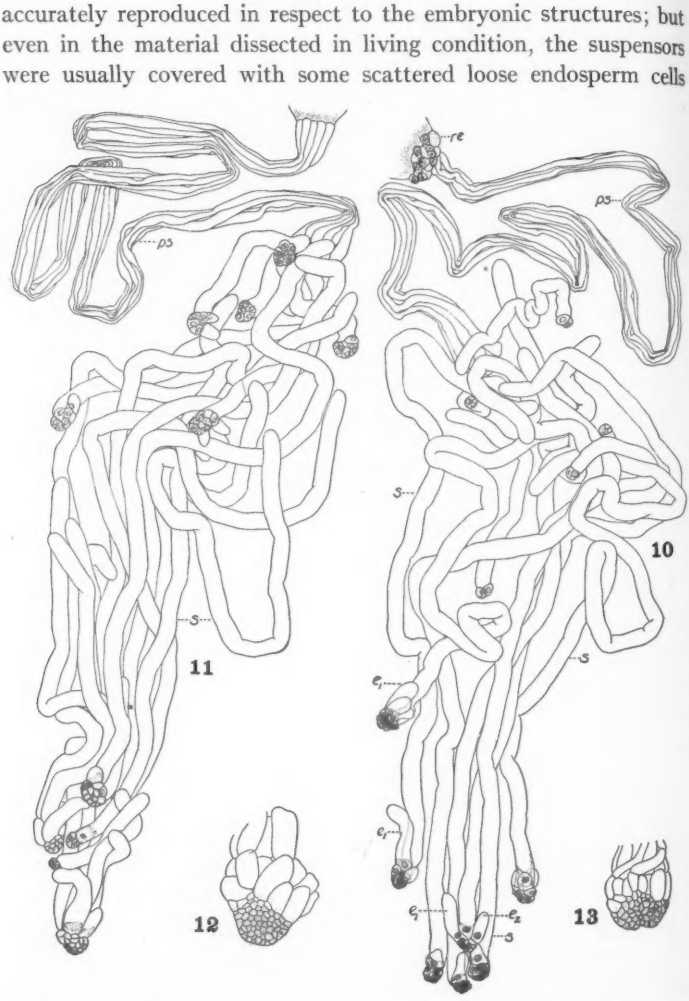
The Kew material, which was collected in June, 1926, contained unfertilized eggs; that of July 2 contained a few embryos of the stages shown in figs. 2 and 3; that of July 9 contained embryos of the stages shown in figs. 10 and 11 and older; and that of July 20 contained embryos of the stages shown in figs. 12–14. This material was killed and preserved for many months before dissection. It came from cones collected at different times and possibly from different trees, which indicates that the stages shown in figs. 7–9 were largely missed, because this stage is passed through rapidly; and the stages of the different collections did not correspond closely. In part, the difficulty was due to unsatisfactory dissections of these particular stages from the ovules of preserved material, with the loss of some of the parts which belong to the same embryo system and should hang together. Aside from the difficulties in the dissection of preserved material, there were so many loose endosperm cells adhering to the suspensors that they were difficult to interpret. In the study of conifer embryos by dissection, it has always been found advantageous to have the living material, although preserved material is sometimes useful when properly killed and fixed. In general, the same conditions were found in the Kew material as in the Salem cone in slightly older stages. The stages represented in figs. 4, 7, and 8 are passed through so rapidly, that in order to make certain to obtain this stage it would probably be necessary to make collections during 3- or 4-day intervals.

The embryos were stained in Delafield's haematoxylin and mounted in diaphane, following the general method described elsewhere (**6**). The drawings were made with a camera lucida, and are



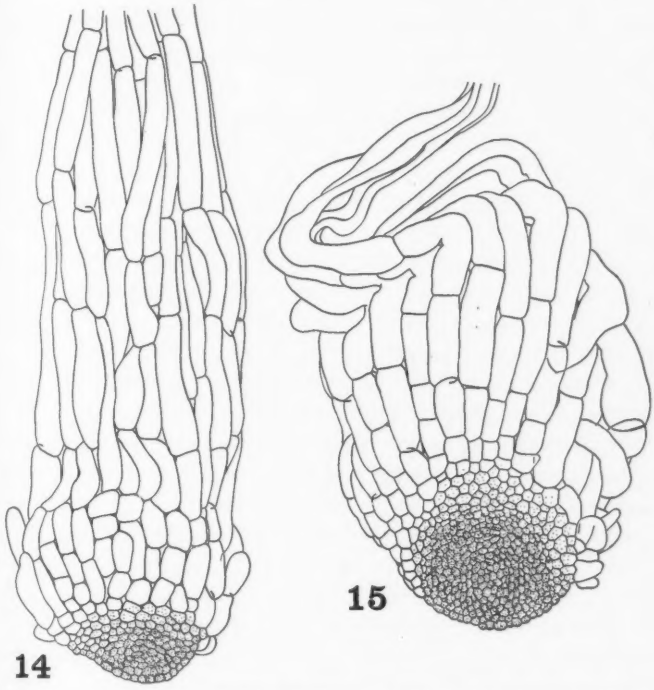
FIGS. 1–9.—Fig. 1, embryo system in earliest observed stage in embryogeny: *r*, group of rosette cells; *ps*, prosuspensor. Fig. 2, embryo system after prosuspensor is fully elongated, with aborted cell below at tip and another at side (only 3 rosette cells found above prosuspensor, central one of which has divided). Fig. 3, lower end of another similar embryo system. Fig. 4, embryo complex composed of two adjacent embryo systems with a few scattered rosette cells above; embryo system at left shows no aborted cells, while in the one at right a dead cap cell has been pushed aside by elongation of a primary suspensor (*s*) of a 2-celled embryo unit. Fig 5, lower end of another embryo system in same stage. Fig. 6, embryo system still attached to archegonium in absence of prosuspensor; several embryos have reached 2-celled stage. Fig. 7, complex of two adjacent embryo systems in which many embryo units are producing primary suspensors while others still remain without elongated primary suspensors, giving appearance of suspensor system in two sections with small cells between. Fig. 8, similar stage in which embryo system at left remains in stage of fig. 2, while the one at right is slightly older than systems of fig. 7 and contains many 3- and 4-celled embryos, on elongating primary suspensors. Fig. 9, embryo system similar to that of fig. 6, with primary suspensors of individual embryo units elongating; ×50.

accurately reproduced in respect to the embryonic structures; but even in the material dissected in living condition, the suspensors were usually covered with some scattered loose endosperm cells



FIGS. 10–13.—Fig. 10, embryo system after primary suspensors (*s*) have become very long and have pushed the collapsed prosuspensor up toward micropyle; rosette embryos (*re*) ready to form primary suspensors; embryonal tubes (e_1 e_2 etc.) beginning to elongate from many embryos. Fig. 11, similar condition in more advanced stage, for embryo system which has no rosette embryos. Figs. 12, 13, successive stages in development of individual embryos after secondary suspensors have become multicellular; ×50.

omitted from the drawings. These could easily be recognized by their position and shape, their starch content, and their multinuclear condition. All drawings are surface views of dissections, and show the structures observed in the highest planes of focus.



FIGS. 14, 15.—Successive stages in development of individual embryos after secondary suspensors have become multicellular; ×75.

Some of the older stages of the embryo have been imbedded for sections. These may be described in greater detail and included in a comparative study, now in progress, of the later stages of various conifer embryos. It is intended here to describe the development of the suspensor and early embryo, including the peculiar conditions of cleavage polyembryony found in this genus. This includes figures of the suspensors similar to those which ARNOLDI described, as well as later stages.

Suspensor formation

Fig. 1 represents the earliest normal stage which could be found in this material. At this stage the characteristic twisting and coiling of suspensor cells found in conifers has begun. This is the beginning of an embryo system which includes all the embryonic parts derived from a single archegonium. The four or five archegonia are terminal, are somewhat deeply imbedded, and are not grouped into an archegonial complex as in the Taxodineae and Cupressineae, although they seem to lie close together. ARNOLDI (1) found an occasional lateral archegonium, while LAWSON (17) did not mention lateral archegonia.

It is evident that the proembryo which was not observed must consist of at least three regions: the lower which includes the group of embryonic cells shown below in fig. 1, the suspensor cells which elongate, and sometimes a group of rosette cells above these. There is probably an additional tier of free nuclei above the rosette, as in some other conifers, which would give a proembryo formed of four tiers. Fig. 1 is already advanced to a stage in which traces of such a group of nuclei would have disappeared.

Not all the embryo systems have a rosette group, as indicated in the rosette region of figs. 7 and 11; in fact, these cells are more commonly absent or reduced to only a few cells, as shown in fig. 4. When the rosette is absent, it is probably the uppermost layer of cells which elongates as a group of suspensor cells. This condition would presuppose fewer tiers in the proembryo, or that more of the embryonic cells were included in the terminal group below. In order to obtain a statistical estimate of the prevalence of rosette cells, 100 embryo systems were examined. Of these, 15 had conspicuous rosettes of many cells, similar to the rosette in figs. 1 and 10; 6 others had similar rosette groups which had nearly disintegrated; 47 had only 1–3 or 4 rosette cells, as in figs. 2 and 4; 29 had no rosette cells at all, as shown in figs. 7 and 11; and 3 systems were found without the large elongated prosuspensor, as shown in figs. 6 and 9.

Elsewhere (6) the writer has found it necessary to allude to the group of suspensor cells (*ps*) which elongate from the proembryo as a "prosuspensor," and it is appropriate to use this terminology here. Likewise it may be less confusing to state at the outset that the

embryo system of fig. 1 is in reality a group of many embryos, mostly in the 1-celled stage. These cells, therefore, are embryo initials, some of which are dividing or have already divided into 2-celled embryos at this stage. The number of lower embryo initials is 12 to 18 or more; the number of cells in the prosuspensor (*ps*) is usually 7 to 9; the rosette cells (*r*) 0 to 9 or more; and the number of free nuclei above the suspensor, if there are any, is unknown.

A number of the embryo initials are sometimes found undergoing disintegration as the prosuspensor becomes elongated. In figs. 1 and 5 all of the embryo initials which are visible appear to be living and active, while figs. 2 and 3 each show a terminal embryo initial which has become transparent and empty. This empty cell is in the position of a cap cell, resembling the cap cell of *Podocarpus spicatus* (**6, 21**) and *Cephalotaxus* (**5, 9, 15, 22**). Fig. 4, which is made up of two embryo systems, appears to be without such a cap cell in the embryo system at the left, while in the system at the right a sterile cell is still visible near the tip. As stated, other cells anywhere in this terminal group may die or become crushed as the prosuspensor is elongated. It is evident, therefore, that through abortion of embryonic cells, the number of embryo-initial groups which are found in later stages may vary considerably.

That the prosuspensor may become exceedingly long is indicated in the figures of the subsequent stages (figs. 2, 4). It becomes greatly coiled and twisted as it pushes the terminal group of embryonic cells deep into the prothallium. No doubt this mechanical action is responsible for the crushing of some of the embryonic cells. A digestive enzyme is present which corrodes the surrounding prothallial tissue, and soon creates a funnel-shaped cavity, flattened slightly and extending downward from the archegonia. This cavity in which the embryos lie is not always clearly bounded in these early stages, for the embryos are frequently found tightly imbedded in the spongy cells, and are not easily removed by dissection; when removed many of the loosely scattered endosperm cells of the gametophyte may remain attached to the surface of the prosuspensors and embryonic cells.

The nuclei of the early embryonic cells (figs. 1–5) are large, and are bounded by a narrow margin of dense cytoplasm, free from

vacuoles. From 2-celled embryo initials individual primary suspensors (*s*) may begin to elongate before the prosuspensors have attained their maximum length. When this elongation begins, each embryo unit may be 2-celled, but is frequently 3- or 4-celled. The system shown at the right in fig. 4 has a terminal embryo, with the upper one of its two cells (*s*) beginning to elongate to form a primary suspensor. Fig. 5 shows a slightly earlier stage in two similarly situated 2-celled embryos, while in figs. 7 and 8 many of the embryos have become 3- or 4-celled. It will be seen that the small embryos are variously oriented and begin to elongate in all directions, but in general they tend to grow downward.

This stage finds the group of embryo initials thrust well down into the prothallium. For a brief period, while the embryo initials have not all produced primary suspensors, the entire suspensor system gives the appearance of a suspensor in two sections with the suspensorless embryonic cells between. These stages are illustrated by figs. 7 and 8, where in each case two embryo systems are shown side by side. It is clear, therefore, that the "small cells between two sections of suspensor" represent embryo initials situated between the prosuspensor and the primary suspensors of slightly more advanced embryonic units.

During the early stages, the individual cells of the prosuspensor have a large single nucleus, usually found in the lower end near the embryo initials. When the prosuspensor has become fully elongated and its cells are coiled and twisted, the nuclei disappear and the prosuspensor soon begins to collapse (fig. 7). Figs. 10 and 11 show the collapsed prosuspensors coiled back in the archegonial region, where they have been crowded into this position by the primary suspensors, as these have elongated upward from individual embryo units in the process of pushing the latter farther and farther downward. When the primary suspensors have become very long, as in fig. 10, some of the other adjacent cells of the embryo begin to elongate, initiating the formation of embryonal tubes which make up the secondary suspensor. As the number of these increases, the secondary suspensor becomes more and more massive (figs. 10–15).

Fig. 6 shows the lower portion of an archegonium in which a prosuspensor did not elongate. The embryonic cells are mostly ad-

vanced to the 2-celled stage, but are still attached to the base of the archegonium. Such conditions were rare, including about 3 per cent of the embryo systems. Fig. 9 represents an older stage similar to fig. 6, in which the embryos were slightly disarranged in mounting. Here are small embryos which, in the absence of a prosuspensor, are beginning to form primary suspensors at the base of an archegonium.

Apical cell growth

The embryos begin to develop by means of apical cell growth, but the apical cell is not always easily recognized, since orientation of the different embryo units varies considerably. The primary suspensor cell appears to be the first segment of the apical cell, which usually places its next wall nearly vertical to the first, and the following wall is inclined to both of the first two. It is probably an apical cell of three cutting faces. After twelve cells have been formed in each embryo, the apical cell with its last few segments may be identified when oriented to afford favorable side views. The embryos found in figs. 10 and 11 still appear to have apical cells, but in the stage shown in figs. 14 and 15 the embryos are advanced beyond the stage in which apical cells may be recognized. A close estimate of the number of cells in an embryo, when the apical cell disappears or is no longer recognizable, was not made, but it is safe to say that the number is much smaller than in *Pinus* (**2**), where as many as 500 cells have been estimated in some embryos before the apical cell disappears.

Polyembryony

Fig. 10 shows an embryo system in which twelve embryos may be counted on long primary suspensors below the collapsed prosuspensor. Those found in the terminal group far below are the largest. They are beginning to form embryonal tubes (e_1 e_2) as secondary suspensor additions, which project backward and may diverge from various sides to form spurs. The number of these projecting spurs increases rapidly as the embryo enlarges, and fig. 11 illustrates how the terminal embryo is gaining the advantage over the others in pushing them back through the use of embryonal tubes. The suspensor becomes more massive by the addition of more and more embryonal tubes (figs. 12–15) which elongate from the

embryo. In fig. 14 the suspensor has gradually become more massive, while in fig. 15 the transition to a massive suspensor is more abrupt. The latter might give the outward appearance of ARNOLDI's fig. 27. I cannot confirm ARNOLDI's statement, however, that all of the product of a zygote may give rise to a single embryo. Each embryo system constantly splits up into many embryos. Obviously each of the small embryos which is beginning to elongate on an individual primary suspensor in figs. 7 and 8 continues independent development. For a time they all appear to be engaged in a struggle against the rest, until one of them gains an advantage and succeeds in pushing the others back toward the micropyle by means of an enlarging secondary suspensor.

Cleavage polyembryony of an extreme type is found in *Sciadopitys*. In spite of the loss of embryo initials through abortion in early stages, more embryos are produced per zygote than in any conifer which the writer has investigated. In *Biota* (**6**) sometimes as many or more embryos may be found in the entire embryo complex, but these represent a tangle of several embryo systems, which were actually contributed by several archegonia. The embryo system shown in fig. 10, which came from a single zygote, has twelve separate embryos, without considering the rosette. Many instances were found in which two or three systems were combined in stages older than figs. 7 and 8, but no attempt was made to illustrate them since they were too complex to be useful in this description.

ROSETTE EMBRYOS.—The rosette cells (*r*) shown in fig. 1 represent still other embryo initials which may begin to divide to form embryos. Fig. 2 shows only one of the three or four rosette cells divided. Fig. 1 shows three out of eight or nine rosette cells which have divided in beginning to form embryos, while the rosette of fig. 10 is in a slightly later stage. A few older stages have been found in which some of the rosette embryos were beginning to put out suspensors; also many more stages in which rosette cells were crushed and aborted. The fact that these cells divide (figs. 1, 10) and occasionally have short suspensors indicates that rosette cells, when present, are groups of embryo initials. If we estimate the number of potential embryos in the rosette from 0 to 9 and the embryo initials below the prosuspensor from 12 to 18, the possible

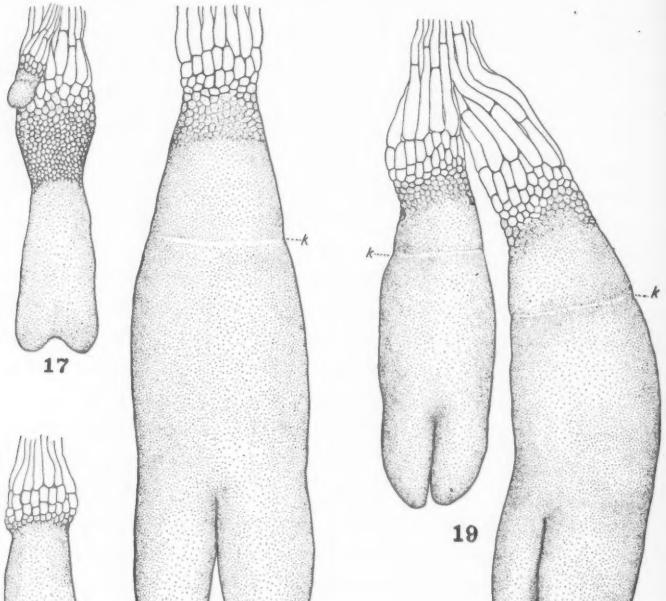
number of embryos from each zygote may reach 20–27 as a maximum, on the basis of the cleavage referable to the early zygote. This maximum estimate may be too high, for it is probable that when a maximum number of embryo initials is found below the prosuspensor, there are fewer of these included in the rosette.

Budding and twinning of embryos.—Following the stages of the embryos shown in fig. 11, they may become deeply lobed and finally divide still farther. Still more embryos may result from a later division of some of the larger multicellular embryos of the lower group. This late division of the embryo is designated as bud formation or budding, in order to distinguish it from the cleavage polyembryony which results from the component parts of the early embryo system. These divisions may be due to a division of the apical cell, resulting in two groups of cells appearing as two unequal or nearly equal lobes. Several cases of budding were observed, although usually the embryos do not form buds but appear as in fig. 12. Fig. 13 shows an instance in which the two lobes were nearly equal in size. Presumably this might result in the development of two embryos if the two should be in the terminal position. In this stage it is still possible to determine that this is a case of budding, since this secondary suspensor of embryonal tubes may be traced back to a common origin from a primary suspensor attached in turn to the remains of the prosuspensor. If two separate embryos had grown together to give this appearance, their secondary suspensors would be expected to separate easily and trace back to separate primary suspensors, showing an independent origin of the two embryos.

Fig. 19 seems to be an instance of twinning which resulted from the development of two bud lobes coming from a division of a single embryo. The two embryos were on the end of a common, closely interwoven secondary suspensor, as indicated by an examination and dissection which was carried out after the drawing was made. The prosuspensor of neighboring zygotes may usually be disentangled and may easily be separated by dissection.

In these later stages, however, when two buds of equal size are attached to the same secondary suspensor, they have an opportunity to develop farther into the later stages, if they occupy a terminal position among the embryos.

Whether formed by this later stage of budding or by the earlier polyembryonic cleavage, the embryos in fig. 19 would probably represent identical twins. Considering the usual slight inequalities of two closely associated embryo systems such as those of figs. 7 and



FIGS. 16–19.—Figs. 16, 17, external appearance of embryos at formation of cotyledons. Fig. 18, mature embryo with two cotyledons, as dissected from seed nearly ready to be shed. Fig. 19, twins dissected from similar seed showing connection by means of common secondary suspensor; *k*, margin of root cap; ×25.

8 (due to unequal development of the prosuspensors), it is unlikely that two embryo systems would contribute two fully developed embryos to the combined polyembryonic complex. There is, therefore, great probability but not absolute certainty that two embryos found matured in the seeds of *Sciadopitys* represent identical twins. Fig. 19 represents a case of identical twins even though they are

unequal in size. When two embryos survive to this stage, it is probable that they both germinate, as they occasionally do in some of the large-seeded pines (**12**). The theoretical possibility of identical twins in conifers may be of interest, but the mechanism of embryonic development is so designed that the survival of more than one embryo is only the rare or exceptional condition. From dissections of about forty embryos in stages between figs. 16 and 18, only one case of two embryos with cotyledons was observed. This is the one shown in fig. 19; however, there were usually many small embryos pushed up above the suspensor of the older embryo. These range in size from the one shown above in fig. 17 to the size of fig. 12 (represented as thrice the magnification of figs. 16–19) and smaller.

Order of differentiation of embryonic tissues

No distinct differentiation of tissues was found, other than that of embryonic cells and the massive secondary suspensor of embryonal tubes in the stages of figs. 11, 12, and 14. Between the stages of figs. 14 and 15, the arched internal arrangement of cells forming the plerome apex of the root becomes recognizable. In *Pinus* (**2**) the stem tip meristem becomes visible soon after the plerome apex of the root, although it develops into only a slight protuberance and is soon followed by a ring of cotyledons surrounding the stem tip. As shown in fig. 17, no such protuberance marking the stem tip meristem appears before the two cotyledons are formed. Embryos of the size represented by figs. 18 and 19 are still without a visibly organized stem tip meristem. The stem tip is probably delayed until after the seed begins to germinate. This condition was found in *Cephalotaxus* (**5**), and differs from all the Abietineae (**2, 3**), where a more or less distinct cone usually marks the meristematic bud of the stem tip, appearing before the cotyledons and long before the embryo is half grown. Two cotyledons are usually formed from as many broad primordia; occasionally three have been found. Out of 36 embryos with cotyledons or well formed primordia, three were found which had three cotyledons. Figs. 18 and 19 show a slight depression (*k*) surrounding the root end of the embryo. This marks the marginal edge of the root cap. *Pinus* does not show such a distinct depression externally, but the internal cell arrangement shows

where the root cap begins, and the root cap embraces about one-half the length of the embryo axis. On the other hand, *Cedrus* and also *Keteleeria* (11) have a shorter, more distinct, and similarly marked root cap which is recognizable in an early stage by the shape of the embryo (3). In *Sciadopitys* the root cap is of much smaller proportions than in *Pinus*. It includes less than one-fourth the length of the embryo, while in *Pinus* it may include nearly half the length of the embryonic axis. In both *Pinus* and *Sciadopitys*, and in conifers generally, so far as has been observed, the root cap is continuous with, and merges gradually into, a massive secondary suspensor. These internal anatomical features will be described elsewhere in greater detail.

Discussion

The embryogeny of *Sciadopitys* represents a somewhat generalized and rather variable type. Contrary to the opinion expressed by LAWSON (17), certain features have been found in the embryogeny which relate it to that of the Taxodineae, Cupressineae, and several other conifer lines. Cleavage polyembryony was the rule from which no exceptions were found in an examination of many embryo systems. The number of embryos formed per zygote greatly exceeds the usual eight embryos of *Pinus* and *Cedrus*, but these three genera do have in common the characteristics of cleavage polyembryony, an apical cell stage in the individual embryos, and rosette cells and embryos. Aside from these features, the embryogeny differs distinctly from that of the Abietineae (2, 4, 7).

The proembryo of *Sciadopitys* undergoes at least one more free nuclear division than the Abietineae before wall formation. According to the partial description of the proembryo given by LAWSON, the terminal group of cells, or the lowest tier as he calls it, organizes into more than twelve cells instead of the four found in the lowest tier of the Abietineae. It may be presumed that these cells represent embryo initials, and that their organization is not completed even after walls begin to appear in the proembryo. In this feature the proembryo differs from that of *Pinus*, where the four primary embryo initials appear to be organized when the first cell walls are laid down, followed immediately by formation of the tier of rosette embryo initials.

The sixteen or more species of *Pinus* which have been studied showed no budding whatever in a later stage. The more mature embryo of *Sciadopitys* also differs from that of *Pinus* in the length of the root cap, the number of cotyledons, and the time of organization of the small protuberance which represents the stem tip. The embryogeny of *Sciadopitys* therefore shows a great divergence from that of *Pinus* in its later stages; and in the early stages it has only the tier of rosette cells, cleavage polyembryony, and the apical cell stage as features in common. The prosuspensor of *Sciadopitys* probably has no corresponding structure in the embryogeny of *Pinus*, except in the rare cases where rosette cells elongate (**2**). The embryonal tubes or additions of secondary suspensor cells in *Pinus* are usually formed differently, since the apical cell has, for a longer period, a single cutting face.

The Cupressineae do not differ greatly from *Sciadopitys* in their embryogeny. If *Biota* and *Libocedrus* (**6**) are somewhat representative of the Cupressineae, the points of agreement embrace such features as the formation of a prosuspensor, but there seem to be no rosette cells. *Biota* differs from *Sciadopitys* in having a relatively long period of primary suspensor elongation during which the terminal cell remains inactive (**6**). In *Sciadopitys* the embryonic cells divide soon after the primary suspensors begin to elongate, and may divide to form several cells before elongation. So far as observed, *Biota* agrees with *Sciadopitys* in the position of the cell walls of the early divisions. It also has budding in the later embryo and usually two cotyledons. The rosette cells of *Sciadopitys* might afford a difference from the conditions in the Cupressineae, as their proembryos and early embryos have been described (**6, 16, 18, 19, 20, 22**), but since *Sciadopitys* was found to vary considerably, having no rosette cells in about one-third of its embryo systems and only a few scattered rosette cells in nearly half, the general conditions may still be considered in essential agreement. The prosuspensor cells of the Cupressineae are fewer in number than in *Sciadopitys*, as are also the number of free nuclei formed in the proembryo. On the other hand, the archegonial complex of the Cupressineae constitutes an organization not found in *Sciadopitys*, and the resulting smaller archegonia may offer a feature with which a reduction in the number

of free nuclear divisions in the proembryo is in some way connected. Likewise the number of embryo initials represented per zygote may have been conditioned by these changes in the transition from a relatively large embryo resembling *Sciadopitys* to a smaller one resembling the Cupressineae. This suggestion is further strengthened by the fact that in the Callitroideae, represented by *Callitris* and *Actinostrobus* (**18, 19, 20**), which were doubtless derived from the Cupressineae, the number of archegonia is increased still further, and the number of free nuclear divisions is reduced to the condition of wall formation after the second division of the zygote. It is obvious that the transition from *Sciadopitys* to certain of the Cupressineae as we know their embryogenies is not difficult. It seems, in fact, a very natural step in the evolution of conifer embryo types.

The relation which holds between the type of embryogeny found in the Taxodineae and *Sciadopitys* is not so obvious at first sight. COKER'S (**8**) investigation shows that cleavage polyembryony is found in *Taxodium*, and that there are no rosette cells. (COKER uses the term rosette to designate the free nuclei in the archegonium above the prosuspensor, but others have used this term in designating a group of cells with cell walls in this position.) His study of *Taxodium* indicates that a brief stage of apical cell growth may be identified in the earliest stages. The prosuspensor cells may become somewhat separated from one another, but appear to persist and grow very long with one or sometimes two embryo initials or cell groups attached to their ends. When embryos become multicellular, an irregular fringe of embryonal tubes appears at their margins, projecting divergently backward on all sides of individual prosuspensors. The primary suspensor appears to be relatively short and sometimes omitted, by which is meant a suspensor cell which becomes elongated singly between prosuspensor and embryo. At least it appears that if the primary suspensor is not omitted, it is short and may not be distinctly recognizable from the other embryonal tubes which follow by elongation in close succession. This interpretation, based in part upon my own unpublished observations of *Taxodium*, is in agreement with COKER'S account, and brings these embryogenies into sufficient agreement with *Sciadopitys* to permit their derivation from this general type. The archegonial complex, which is also

found here, has probably conditioned this transition to the Taxodineae much as this structure and the smaller archegonia seem to have influenced the transition to the Cupressineae: fewer free nuclear divisions in the proembryo, fewer embryo initials per zygote, and fewer cells in the prosuspensor.

Passing to *Sequoia* (**1, 13**), which appears to stand higher and is placed in a separate subfamily by some botanists, we seem to have smaller and still more numerous archegonia, with no free nuclei in the proembryo, and only a few embryo initials organized so far as known, with only one of them remaining functional on the end of a single-celled prosuspensor. That this parallelism in the evolution of embryogenies has come about in the two lines Cupressineae-Callitroideae and Taxodineae-Sequoideae, where a terminal archegonial complex appeared which involved more and more eggs and finally became lateral, is an extremely interesting situation, which calls for further consideration as other embryogenies in these two groups are investigated. It seems obvious that events connected with the history of the archegonial complex and resultant reduction in the archegonial size have had something to do with the decrease in the number of free nuclei in the proembryo, the reduction in the number of embryo initials per zygote, and the number of cells in the prosuspensor.

In its appearance and organization, the early embryo of *Sciadopitys* relates itself satisfactorily to that of *Cephalotaxus* (**5, 9, 15, 22**). The rosette group and terminal dead cap cell of *Sciadopitys* (figs. 1–5) might be substituted for the embryo of *Cephalotaxus*. The cap cells of the latter are larger, and usually several aborted cells are involved in the cap; otherwise there is no essential difference. In *Cephalotaxus* all embryo initials represented below the prosuspensor combine to form a single embryo, but the rosette cells form small separate embryos which have suspensors of embryonal tubes but no primary suspensor (**5**). An important essential difference between these two types is therefore to be recognized in cleavage polyembryony. In *Sciadopitys*, 12–15 embryos may come from the group below the prosuspensor, one of which contributes the embryo of the mature seed; in *Cephalotaxus* the lower units combine and only a single embryo comes from this region, and those of the rosette region

are more constantly present, persist longer, and develop to an older stage than do those in *Sciadopitys*. Another essential difference between these two types is in the apical cell growth. In *Sciadopitys* apical cell growth may be recognized; in *Cephalotaxus* an apical cell has not been observed, either in the single primary embryo or in the rosette embryos. A difference may possibly be found also in the time of wall formation in the proembryo, since it is not certainly known for *Sciadopitys* whether only eight or more than eight free nuclei are formed before walls appear in the proembryo (**17**).

Podocarpus spicatus has an early embryo which resembles that of *Sciadopitys*, at least in general organization (**6, 21**). In *P. spicatus*, however, in common with all species of *Podocarpus*, *Dacrydium*, and *Phyllocladus*, binucleate cells are found which represent embryo initials (**6**). Rosette cells which early become aborted are sometimes present, and the terminal binucleate cap is usually a single cell. While *P. spicatus* otherwise resembles *Cephalotaxus*, it has more completely eliminated cleavage polyembryony, producing only a single embryo from the group of cells below the prosuspensor, and no rosette embryos from the few scattered rosette cells. It appears, therefore, that *Sciadopitys* has an early embryogeny which may be considered generalized rather than specialized. It may serve to illustrate a starting point for the embryogenies of both the Taxodineae and Cupressineae. Before cleavage of the zygote, it is so similar in general organization and appearance to the embryos of *Cephalotaxus* and certain podocarps as to suggest a common origin, and it shares several features in common with the embryogeny of *Pinus* and the Abietineae, to which it is more remotely related. This suggests that *Sciadopitys* occupies a central position in the phylogeny of certain groups of higher conifers, remaining near the plexus of several diverging lines of evolution.

Summary

1. The early embryo of *Sciadopitys* shows two or three regions or tiers of cells which may develop into later stages. The cells of one of these regions elongate to form the prosuspensor; those of another represent embryo initials situated below the prosuspensor; and those of the third, which may be present, are rosette cells above the prosuspensor. A cap cell consisting of a terminal aborted embryo

initial may be present, and other embryonic cells anywhere in the embryo system may become aborted.

2. As the prosuspensor cells become fully elongated, the embryo initials give rise to embryos which are pushed out on single-celled, primary suspensors. The early embryos pass through a stage in which an apical cell is recognizable. Rosette cells, when present, are embryo initials. These may give rise to embryos which are usually aborted in early stages.

3. Embryonal tubes are formed which reinforce the primary suspensor, thus giving rise to a multicellular secondary suspensor.

4. The potential output per archegonium is 12–28 embryos, which may develop independently for a time. If three eggs are fertilized, an embryo complex may be formed with several times this number of separate embryos.

5. Embryos may undergo budding and twinning in later stages; however, the seed usually contains only a single embryo which is fully matured with cotyledons. The number of cotyledons is usually two but occasionally three.

6. The stem tip is not organized until long after the cotyledons are formed.

7. The root cap is relatively short, and merges very gradually into the secondary suspensor.

8. The embryo of *Sciadopitys* is a type from which the embryos of the Cupressineae may have been derived; it also represents a type from which the embryos of the Taxodineae may have been derived. It has probably had a common origin with the embryo type represented by *Cephalotaxus* and certain podocarps, and appears to be more distantly related to the Abietineae.

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AVAILABLE SOIL CALCIUM IN RELATION TO "DAMPING OFF" OF SOY BEAN SEEDLINGS

W. A. ALBRECHT AND HANS JENNY

(WITH TEN FIGURES)

Introduction

The prevalence of "damping off,"[1] with its large toll of plant seedlings, and even of plants, makes it a disease so serious as to invite study of any plausible suggestion toward its prevention. The plants which may be affected are not limited to any species, genera, or even family. STEVENS and HALL (9) state that any kind of plant is susceptible. Fungi have commonly been considered the pathological agency, with emphasis on *Pythium de Baryanum* and *Rhizoctonia* (6), although the list includes *Fusarium*, *Botrytis*, *Corticium*, *Thielavia*, *Sclerotium*, *Phytophthora* (9), and numerous others. Attention has been given to environmental conditions as factors (4, 9, 5), placing importance on high temperature and high moisture as favoring development of the disease. The soil has been considered as the habitat for the infecting microorganisms, but, so far as known, no attention has been directed to the possibility of the disease being associated with the physico-chemical properties of the soil.

In some studies attempting to isolate the effects of the soil calcium from those of the hydrogen-ion concentration upon inoculation and growth of the soy bean, the damping off of the seedlings occurred frequently in spite of complete sterilization of the seeds and the media. Variation in temperature or moisture conditions, the correction of crowding, the use of artificial ventilation, outdoor growth, and the exclusion of organic matter from the medium, all failed to prevent damping off. These facts, coupled with our recent knowledge of soil

[1] In this study, the term damping off refers to the dropping over of the young plant and other readily visible symptoms associated with the disease usually designated by that name. No study of the infecting fungi, internal plant structure, or other conditions was made. No claim is advanced that the disease in this study is the exact disease of this name by other investigators. For this reason throughout this paper the term damping off should be read as in quotation marks.

colloidal behavior (**1, 8**), suggested the hypothesis that physico-chemical conditions centering around the calcium and hydrogen-ion concentrations of the soil might also be responsible for this disease.

Methods

The plan of testing calcium and pH as factors influencing damping off necessitated careful control of the physico-chemical conditions of the soil or substrate. As a growth medium, a mixture of quartz sand, calcium clay, and other calcium compounds was used. The quartz sand was first treated with strong hydrochloric acid and then extracted with water until free from chlorides and until neutral to bromthymol blue. A natural clay was obtained from the fresh subsoil of a Putnam silt loam by separating out the colloidal fraction by means of the super centrifuge. The size of the particles of this clay varied, according to BAVER (**2**), from 121 to 136 mμ. Analysis showed that it contained primarily adsorbed calcium and hydrogen ions, and that it had a molecular ratio of silica to alumina of about 3 to 1.

In order to eliminate unknown variables in the composition of the clay, this material was subjected to rigid purification by electrodialysis, according to the method of BRADFIELD (**3**). This method removes particularly those ions which are adsorbed[2] in exchangeable form on the surface of the clay particle. Clay so prepared contains for practical consideration only H-ions in exchangeable form. It is acid to a degree as low as pH 3.5, depending on the clay-water ratio, and permits varying degrees of substitution of the adsorbed H-ion by any other cations. These are also held by the clay particle in exchangeable form. The entire system is well buffered.

For the preparation of the calcium clay, varying quantities of calcium hydroxide were added to the pure hydrogen clay. The changes in degree of acidity in consequence of this treatment, as determined by the hydrogen electrode, are given in the titration curve of fig. 1. This curve served as the basis for the preparation of clays of varying hydrogen-calcium ratios, and consequently of vary-

[2] In using the term adsorption, it is intended merely to state that the ions are held on the surface of the colloidal particles; no attempt is made to distinguish between the chemical or physical nature of the forces responsible.

ing amounts of exchangeable calcium. Clay so prepared was mixed with sand in proportions never exceeding 2 per cent clay, and afforded excellent textural conditions.

Since adsorbed calcium enters into solution only when exchanged or replaced by other cations (including H-ions), it was deemed advisable to compare this clay-calcium compound with solutions having free, diffusible ionic calcium of the same concentration. Calcium acetate and calcium chloride were used for this comparison. Calcium acetate of various degrees of acidity was prepared according to the titration curve in fig. 1. No other nutrients were added to the media, since colloidal soil studies have demonstrated that the addition of ions to colloidal clay brings about, through ionic exchange and adsorption, entirely new conditions (7). The plants were limited to the nutrients in the seed and the media. This method of using the clay to supply the calcium limited the amount of this element to that quantity released from the clay by plant action.

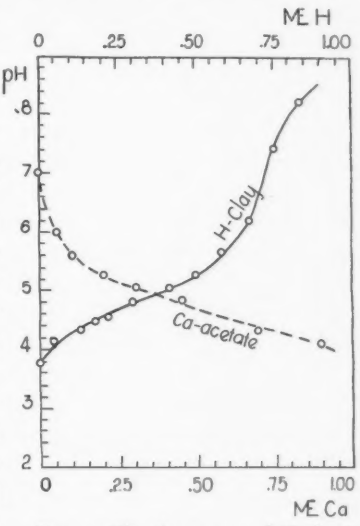


FIG. 1.—Titration curves of H clay+calcium hydroxide and Ca acetate+acetic acid as bases for preparation of media (ME Ca and ME H, milliequivalents of calcium and hydrogen, respectively).

Investigation of damping off

SYMPTOMS.—The symptoms of damping off as noted were a browning of the stem area just above the sand surface, the stem becoming soft, flaccid, and slimy, with a final breaking over of the plant. In some cases the degree of browning was less marked, the plant remained standing, became stunted, withered at the top, developed many adventitious buds at the cotyledons, and later the stem above this point fell over. After the first signs of the disease,

counts of the afflicted plants were taken at regular intervals until time of harvest.

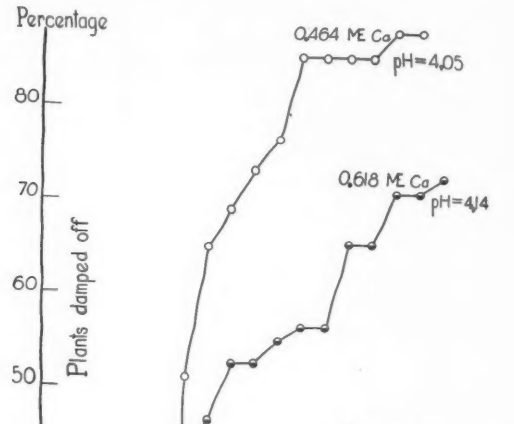


FIG. 2.—Increase of damping off with time: upper curve, low calcium content (0.464 ME per 65 plants) highly acidic (pH 4.05); middle curve, higher calcium content (0.618 ME per 65 plants) less acidic (pH 4.14); lower curve, high calcium content (1.396 ME per 65 plants) neutral reaction (pH 6.94).

RELATION TO TIME.—In all cases investigated it was found that damping off showed a definite relation to time (fig. 2). After germi-

nation between moist towels (root tips being 1–3 cm. long), the seedlings were transferred to glass jars with sandy medium. The plants grew vigorously for a few days, but after a week damping off occurred very noticeably, in that a great number of plants became afflicted suddenly and died within a short time. With increasing time, the rate of damping off gradually lessened, bringing about a curve which makes an asymptotic approach to the maximum of damping off for each particular treatment.

EFFECT OF VARIATION OF BOTH CALCIUM AND HYDROGEN IONS.—In order to simulate conditions of natural clays of humid regions, which contain primarily calcium and hydrogen ions in the adsorption complex, the first part of the study used constant amounts of clay but varied the amount of calcium and hydrogen ions on the clay particles. For every increase in calcium, there occurred a corresponding decrease in acidity (hydrogen ions). Two different experiments were run, extending from May 2 to July 15, and from July 10 to August 10, 1929. Glass jars with 1000 gm. of sand, each containing the necessary amounts of 1 per cent clay sol in one series and 0.052 normal calcium-acetate solutions in another series (for comparison), were planted each with 65 sterilized soy bean seeds. The water content of 20 per cent was maintained constant by daily weighings and water additions. Glass tubes were inserted vertically to facilitate aeration. Other environmental conditions were maintained as uniform as possible under greenhouse conditions.

The first experiment increased the calcium in colloidal clay and as calcium acetate from 0 to 2.15×10^{-2} milliequivalents per plant through ten jars by increments of approximately 0.24×10^{-2}, and decreased the hydrogen correspondingly from 2.3 to 0.15×10^{-2} through a pH range from 3.78 to 6.94. The results of these trials are given in fig. 3. In the second experiment, in order to use larger amounts of calcium, it was increased in these same two forms from 2.3 to 20.9×10^{-2} milliequivalents per plant through five jars by increments of approximately 4.6×10^{-2}, while the hydrogen decreased from 18.6 to 0×10^{-2} through a pH range from 3.8 to 8.0. Here again the clay series suffered more damping off than the acetate series. The range of the affliction was from 29 to 5 per cent for the clay series and from 14 to 0 per cent for the acetate series, decreasing in each with increasing calcium.

The most noticeable result of these two trials is the fact that, within the range in amounts of calcium and hydrogen ions used, the damping off decreased as acidity decreased and calcium increased. This is so, as shown in fig. 3, whether the calcium is supplied in adsorbed form by calcium clay, or in ionic form as calcium acetate. The percentage of injured plants was lower for calcium acetate than

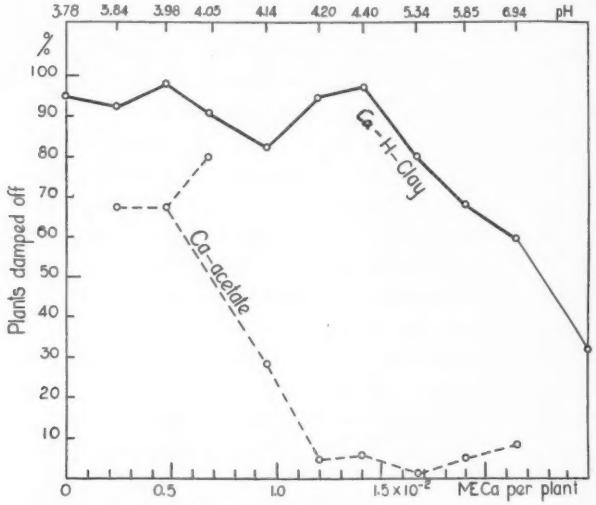


FIG. 3.—Decrease of damping off with falling acidity and increasing calcium concentration.

for calcium clay (fig. 4). This difference was less marked, however, and tended to disappear, as the ratio of hydrogen ions to calcium ions approached zero (fig. 3). It is interesting to note that in the second experiment, where more clay and consequently more hydrogen and calcium ions per plant were supplied, the percentage of damping off at any pH was considerably less than in the first trial, although in both cases the hydrogen-calcium ratio remained unchanged. The main significance of these trials undoubtedly lies in the fact that the observed damping off is definitely associated with the nature of the cation adsorbed on the colloidal complex.

Since the decrease of damping off resulted from variations in

amounts of both hydrogen ions and calcium, the causal effect cannot be specifically ascribed to either the calcium deficiency or the hydrogen-ion excess. In order to isolate the specific influence of the two variables (Ca and H), it was necessary that one factor be kept strictly constant while the other was varied.

Effect of hydrogen ions on damping off at constant calcium concentration

I. Low calcium concentration.—In the third trial an attempt was made to isolate the separate effects by the hydrogen ions on

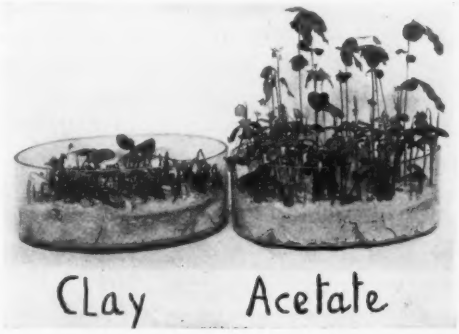


Fig. 4.—Damping off more pronounced with Ca clay than with Ca acetate (pH 4.05, Ca concentration 0.72×10^{-2} ME Ca per plant in both jars).

damping off. Calcium was kept constant but at a low concentration. Recent physico-chemical investigations of soils have shown that the colloidal clay fraction has a certain saturation capacity for cations. Consequently the total ions to be adsorbed and their ratio to each other are determined by this saturation capacity. There is then an upper limit in the adsorption of calcium for a given amount of clay. Below this limit the amount of calcium may be varied, but it is accompanied by an inverse variation in the hydrogen, or gives a varying hydrogen-calcium ratio. In order to increase the hydrogen-ion content of the medium beyond this limit, larger amounts of clay must be added.

A series of nine pots was used, containing in every case the constant amount of 0.0146 milliequivalents of calcium per seed in the

form of calcium clay. The acidity varied from pH 3.8 to 6.94. In order to obtain the necessary constant calcium concentration at low pH (wide H:Ca-ratio on the clay particle), larger amounts of clay per seed had to be added to the sand. The amounts did not exceed 2 per cent of clay in the sand. These trials extended over the same time as the preceding ones and were conducted under similar care and conditions. The data of the numbers of diseased plants for the various pH figures are given in the upper graph of fig. 5.

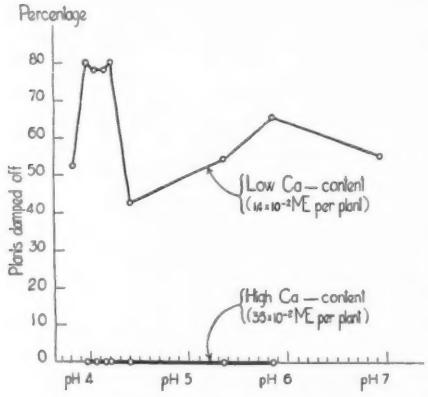


FIG. 5.—Negative effect of pH of Ca clay on damping off in presence of low and high calcium content.

For these low amounts of calcium, no definite correlation was observed between the percentage of diseased plants and clay acidity. The number of dead plants varied from 43 to 80 per cent, irrespective of whether the reaction of the medium was neutral or acid. Even near pH 7.0, where the hydrogen-calcium ratio approaches zero, over 50 per cent of the plants died. This suggests that under constant, low calcium concentration the disease is not controlled by the amount of hydrogen ions present. In this trial with low calcium application there was a marked manifestation of the disease independent of the hydrogen-ion concentration. This, however, does not necessarily guarantee the indifference of the disease to the hydrogen-ion concentration at other concentrations of calcium, nor does it establish a causal relation between the concentration of calcium and the disease.

II. HIGH CALCIUM CONCENTRATION.—In the next trial, a higher concentration of calcium, 0.350 milliequivalents of calcium per seed or an increase of 24 times, and a varying acidity of pH 4–6 were used. This was done by using clay suspensions like the previous,

but by supplying in every case larger amounts of clay. Each plant was grown in an individual container (300 gm. sand) in order to prevent the use of mineral excretions from dying plants by those remaining healthy. Each concentration of calcium was replicated three to ten times. This trial extended from November 22 to January 10, under conditions and manipulation corresponding to those of the preceding trials. The plants were observed daily for the possible development of the diseased condition. The records are given in the lower graph of fig. 5, and may be compared with the data for lower Ca concentrations in the upper graph.

Again at this particular calcium concentration, there was evident no relation between the disease and the hydrogen-ion concentration. But, contrary to the previous experiment at low Ca concentration, not a single plant showed any indication of the disease. This indicates clearly that the hydrogen-calcium ratio (pH) is not a factor in the prevalence of the disease when the supply of calcium is either low or high. It suggests, however, that the disease is related to the second variable, namely, the calcium.

Effect of calcium on damping off at constant pH

In order to test the importance of calcium, there was set up a series of increasing clay concentrations and increasing calcium acetate concentrations, and therefore increasing amounts of calcium per seed, at constant but at two different hydrogen-calcium ratios. One set of plants was grown at about neutral reaction (pH 6.92 and 6.94) and one at acid reaction (pH 4.4).

I. Increasing calcium concentration at neutral reaction.—One series of eight pans contained 65 plants per pan with variations of calcium from 0 to 4.2×10^{-2} ME Ca per seed, all at pH 6.94. The results are given in fig. 6. This experiment was carried out during the summer months of 1929. Another series, with 200 plants grown individually in nursery bottles containing a range from 0 to 35×10^{-2} ME Ca per plant at pH 6.92 lasted from March 15 to May 15, 1930, and gave the results according to table I.

These results, and those shown in figs. 6 and 7, are apparently conclusive as to the importance of calcium in connection with damping off in these trials. With increasing Ca concentration, the number

of diseased plants decreased in the case of both Ca clay and Ca acetate.

TABLE I

DAMPING OFF AS INFLUENCED BY INCREASING AMOUNTS OF CALCIUM AT NEUTRAL REACTION (pH 6.92); SUMMARY OF 200 PLANTS GROWN INDIVIDUALLY

MILLIEQUIVALENTS Ca PER PLANT	PERCENTAGE PLANTS DAMPED OFF
Ca-clay 0	100
$0.5-5\times10^{-2}$	29
$6-20\times10^{-2}$	7
$25-35\times10^{-2}$	3
Ca-acetate 0	100
$5-10\times10^{-2}$	25
30×10^{-2}	0

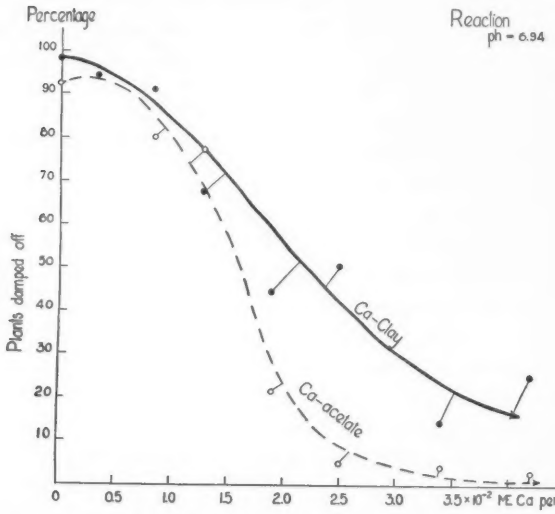
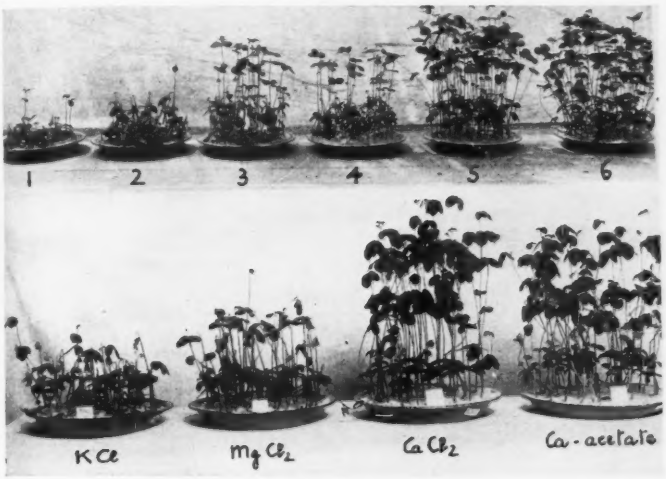


FIG. 6.—Decrease of damping off with increasing Ca concentration at neutral reaction.

II. INCREASING CALCIUM CONCENTRATION AT ACID REACTION.—If calcium is a more important factor than acidity in controlling damping off, the preceding experiment should give similar results at

relatively low pH figures (acid reaction), and not only at pH values near the neutral point. A trial with increasing amounts of Ca (0.86–3.08 ME$\times 10^{-2}$ per plant) at the very acid reaction of pH 4.4 was run during the months of June and July, 1930. The results are given in table II, and show that even at low pH the diseased plants decrease rapidly with increasing calcium concentration.



FIGS. 7, 8.—Fig. 7 (above), decrease of damping off with increasing Ca-acetate concentrations (0–3.4$\times 10^{-2}$ ME Ca per plant) at pH 7. Fig. 8 (below), effect of Ca as compared with that of K and Mg on damping off:

KCl:	10.8$\times 10^{-2}$ ME K per plant
$MgCl_2$:	7.8$\times 10^{-2}$ ME Mg per plant
$CaCl_2$:	8.4$\times 10^{-2}$ ME Ca per plant
Ca acetate:	11.4$\times 10^{-2}$ ME Ca per plant

In summarizing the preceding trials, the first observation, that damping off decreases as the hydrogen-ion concentration decreases and the calcium concentration increases, can be interpreted as follows: calcium ions are far more effective than hydrogen ions in controlling damping off. Trials with varying pH values at constant calcium concentrations (high and low) did not reveal any correlation between the disease and the hydrogen-ion concentration. There was observed, however, a close relationship between calcium content and

affected plants. This relation manifested itself independently of pH values.

Comparative importance of calcium and other cations

In the preceding trials, the effect of calcium in controlling damping off was definitely related to the quantity of calcium present, when the medium contained only calcium and hydrogen. Not only the quantitative effect but also the qualitative importance of calcium in comparison with other cations can be demonstrated. Series of jars of 50 plants per 800 gm. sand were set up, one each for varying amounts of potassium chloride, magnesium chloride, calcium chloride, and calcium acetate. These were handled in the same manner as in previous experiments.

TABLE II

Decrease of damping off with increasing calcium clay concentration at pH 4.4

	Laboratory number		
	1 (a, b)	2 (a, b)	3 (a, b)
Number of plants per pan	65.0	65.0	65.0
pH (initial)	4.4	4.4	4.4
ME Ca$\times 10^{-2}$ per plant	0.86	1.92	3.08
Percentage damped off after 14 days	40	8	0

For all salts used there was a decrease in damping off with increasing amounts of each salt. This decrease, however, was characteristic of each salt. For potassium chloride, the injured plants decreased from 94 to 74 per cent; in the case of magnesium chloride, the change was from 96 to 12 per cent; while for the calcium chloride and the calcium acetate the decrease dropped suddenly from 89 to 0 per cent as soon as a certain minimum concentration of calcium was reached. This minimum occurred at 2–3$\times 10^{-2}$ milliequivalents of calcium per seed. The difference in effects shown by calcium over that by the other cations is illustrated in figs. 8 and 9.

The trials thus far emphasize the significance of calcium in connection with the occurrence of the disease. This significance is attached, first to the element calcium itself, then to the quantity of

calcium present, and finally to the nature of the anion with which it is associated (acetate, chlorine, and "clay"). The experiment also indicates that osmotic pressure of the medium is scarcely connected with the disease.

Availability of calcium and its relation to the disease

The observation that calcium acetate is somewhat superior to calcium clay in preventing damping off, suggests that the availabil-

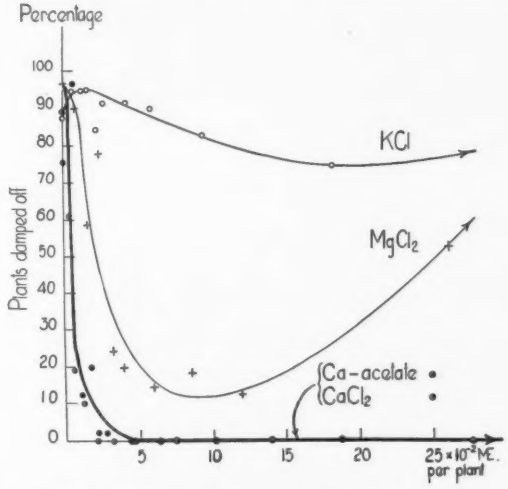


Fig. 9.—Specific effect of calcium in preventing damping off as superior to that of potassium and magnesium.

ity of calcium might also be of importance in controlling the disease. The following Ca carriers were selected to test the validity of this suggestion: (1) calcium acetate, which is highly dissociated and provides calcium in free, diffusable ionic form; (2) calcium permutit, in which case a commercial sodium permutit (an artificial aluminosilicate) was transformed into calcium permutit through continuous leaching with Ca chloride, providing calcium in adsorbed exchangeable form, that is to say, it can be set free through ionic exchange with other cations, particularly hydrogen ions (7); and (3) anorthite,

which is a natural alumino-silicate mineral, insoluble in either hydrochloric or sulphuric acid. Its calcium is held firmly in the crystal through the forces of the crystal lattice.

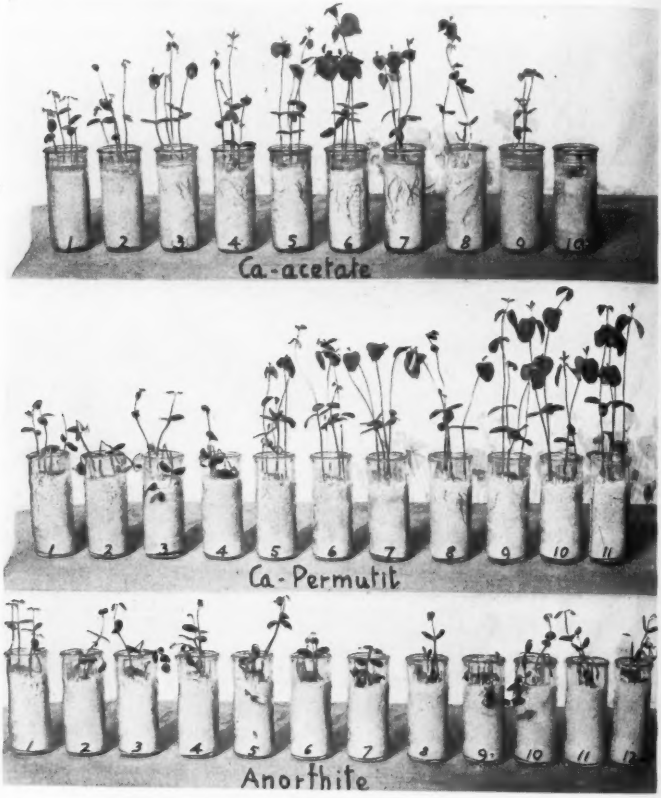


FIG. 10.—Availability of calcium as factor controlling damping off: free, adsorbed, and crystal lattice calcium from upper to lower pictures; increasing calcium from left to right.

Plants were grown in nursery bottles in duplicate series containing 300 gm. of sand each and increasing amounts of calcium, varying from 1.25 to 250×10^{-2} ME Ca per plant (pH 7). Anorthite and permutit were ground and passed through a 40-mesh and collected

on a 60-mesh sieve in order to obtain particles of uniform comparable size. The treatment and results are shown in table III. Fig. 10 indicates the conditions after the tenth day of growth. The results obtained in these trials are striking. The plants grown on calcium acetate showed no signs of damping off; those on calcium permutit damped off at the lower but not at the higher concentrations; while the anorthitic lot was affected by the disease at all concentrations.

TABLE III

AVAILABILITY OF CALCIUM AS IT AFFECTS DAMPING OFF

	LABORATORY NUMBER											
	1	2	3	4	5	6	7	8	9	10	11	12
ME Ca$\times 10^{-2}$ per plant.	0	1.25	2.5	3.75	5.0	7.0	12.5	17.5	25	125	250	1250
pH (initial)	7	7	7	7	7	7	7	7	7	7	7	7
Number of plants per bottle	4	4	4	4	4	4	4	4	4	4	4	4
Number damped off after 14 days:												
Ca acetate	1	0	0	0	0	0	0	0	0	0	....	....
Ca permutit	1	3	2	3	3	0	0	0	0	0	0	....
Anorthite	0	3	3	2	2	2	3	2	4	3	2	2

Evidently the availability of calcium is also a vital factor in the frequency of the disease. The effectiveness of ionic calcium in its power to influence the disease might be represented by the following series:

$$\text{free, diffusable Ca} > \text{adsorbed, exchangeable, Ca} > \frac{\text{fixed Ca in the}}{\text{crystal lattice.}}$$

Summary

1. The damping off disease has usually been associated with unfavorable temperature, moisture, and light conditions, but these experiments show that, in the case of soy bean seedlings, the disease is also closely associated with the nature of soil colloids, particularly with the nature of the adsorbed ions (calcium).

2. Hydrogen ions are not an important factor in determining the disease. Within a range of pH 3.8–6.94 it was found that heavy damping off occurred when the calcium supply was low, but none occurred when the calcium supply was high.

3. Calcium is an important factor in the control of the disease. With increasing calcium concentration the number of diseased plants decreased rapidly, both at pH 7 and at pH 4.4. At high calcium concentration no damping off occurred between pH 3.8 and 8.0.

4. In preventing the disease, calcium ions are superior to other monovalent or divalent ions at equal concentrations. For the chlorides, the importance of cations in checking the disease decreases according to the series $Ca > Mg > K$.

5. Availability of calcium is an important feature in controlling damping off. Free, diffusable calcium ions were more effective than adsorbed, exchangeable calcium ions, while calcium bound by the forces of the crystal lattice in the mineral anorthite was not able to prevent the disease.

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CICATRIZATION OF FOLIAGE LEAVES

II. WOUND RESPONSES OF CERTAIN BROAD-LEAVED EVERGREENS

ROBERT B. WYLIE

(WITH SEVEN FIGURES)

Introduction

A recent article by the writer discussed the foliar wound responses of certain mesophytic plants and reviewed the more important literature in this field. This paper (3) dealt with the leaf organization and described both the preliminary and the secondary protective structures developed by wounded leaves of *Vitis vulpina*, *Rhus glabra*, and *Syringa vulgaris*. In these forms, as in all other mesophytes studied, there is a prompt collapse of dead tissues along the wounded margin following lesion, and the shrunken mesophyll, with the incurved epidermal layers, constitutes a protective buffer which has been termed the pseudocicatrice (2). Subsequent divisions of living cells underneath this preliminary barrier lead to the formation of the cicatrice proper, or specialized protective structure. This latter, which is developed slowly by the modification of living cells, consists in part at least of wound cork and is usually several layers of cells in thickness.

The present paper summarizes events following wounding of certain broad-leaved evergreens of the Pacific northwest. In 1925 the writer made preliminary observations on some of the evergreen angiosperms of western Washington. Further material was supplied by members of the botany staff of the University of Washington, in Seattle. The writer is under special obligation to Professor GEORGE B. RIGG of that institution for collections made in three successive years. During the summer of 1929 the writer spent several weeks at the Puget Sound Biological Station on San Juan Island, 60 miles north of Seattle, where certain experiments were carried out and further material was collected. This latter region is somewhat drier in summer than the Seattle area, precipitation being influenced by ad-

jacent mountains. The winters in the Puget Sound district are moist and relatively mild.

Following a preliminary study of a number of native broad-leaved evergreens of the Pacific northwest, three species were chosen for special study, Madrona (*Arbutus menziesii*), Salal (*Gaultheria shallon*), and Oregon grape (*Berberis nervosa*). These are all deep-rooted trees or shrubs with evergreen leaves which meet the problems of the changing seasons and function through two or more summers. Healthy leaves of typical plants were wounded during the latter part of June by cutting with scissors across the blades at right angles to major veins, and subsequently material was collected at intervals and preserved for critical study. Freehand and freezing microtome sections yielded general information, but for detailed study the tissues were imbedded in paraffin after preliminary treatment with hydrofluoric acid. Serial sections, cut both transversely to and parallel with the leaf surface, gave preparations for detailed study. The latter sections were especially helpful in interpreting the normal leaf structure as well as changes that follow wounds.

I. Arbutus menziesii

This peculiar tree with its beautiful foliage is one of the characteristic plants of the open shores from northern California to British Columbia. Each set of leaves lives somewhat more than a year in the Puget Sound region, where leaf fall occurs during the second summer (1). The oval leaves, with glossy upper surface, are about 7.5 × 12.5 cm. in size, and average 285 μ in thickness.

Transverse sections show a single upper epidermis with no suggestion of special subdermal development (fig. 1). In addition to a heavy cuticle, all walls of the epidermal cells are thickened, but numerous thinner areas in their lateral walls favor transfer of material within this layer. The upper cuticle is 3–4 μ in thickness and about twice that on the under side, which has numerous protuberances that are almost spinous. The lower epidermis has much smaller cells and carries all of the stomata. The inner walls of the lower epidermis have also a well developed cuticle bordering on the large air spaces within the leaf.

The palisade is multiseriate, having usually two specialized layers of cells, while a third layer approaches mesophyll and is somewhat

FIGS. 1, 2, 4.*—Fig. 1, transverse section of *Arbutus menziesii* 40 days after wounding, showing pseudocicatrice (collapsed), cicatrice, and normal leaf tissue; fig. 2, transverse section of *Gaultheria shallon* 40 days after wounding, showing pseudocicatrice, cicatrice, and normal leaf tissue; fig. 4, transverse section of *Berberis nervosa* 40 days after wounding of mature leaf, showing normal leaf tissue, a few enlarged cells, and wide zone of dead tissue with fungal hyphae and spores (there has been no collapse of the dead tissue which occupies three-fifths of this figure).

* All figures except fig. 3 were prepared by using photographs as foundations for drawings. Photomicrographic negatives were made using a Carl Zeiss no. 20, 8.3 mm. apochromatic objective in combination with a no. 1 Homal. From these negatives enlargements were made, which were completed as drawings with microscopic study of the original sections.

intermediate both in position and character. The uppermost palisade layer, seen in section parallel with the leaf surface, has polygonal cells with small intercellular spaces at their angles. The second layer has cells more nearly cylindrical with proportionately larger intercellular spaces, while the third layer is still less compact with much more open space.

The loose spongy mesophyll resembles the palisade in that its cells are mainly vertical in arrangement, in contrast to the usual horizontal trend of cells in this zone. Sections parallel with the epidermis show some cell elongation in this direction, with a loose mesh arrangement only in the lower part of the leaf adjacent to the epidermis. The minor venation is rather uniformly distributed in the form of rectangular meshes which lie in the spongy mesophyll. The intervascular interval averages 227 μ.

Wound responses

The gradual collapse of dead mesophyll cells along the wounded margin leads to the development of a pronounced cushion, often 30 or more cells in depth. In addition to the partial barrier produced by this dead tissue, both upper and lower epidermal layers are drawn inward over the wounded margin of the leaf (fig. 1). This pseudocicatrice develops slowly through a period of several days but begins early its protective function. Subsequent enlargement of living cells underneath may separate somewhat the incurved epidermal layers, owing to the bulging growth of the cicatrice tissues.

Mitoses in living cells underneath the pseudocicatrice seem not to begin until about the tenth day following lesion. By the fifteenth day, the developing cicatrice involves a zone 4–6 cells across, and it is obvious that all cell layers of the leaf have contributed toward its formation through mitoses. A month or more is necessary for completion of the cicatrice. At maturity this barrier involves 6–10-cell layers of wound cork, and underneath are about as many more layers of living cells crowded irregularly together. The outer cicatrice cells enlarge considerably and have heavy walls on the outer convex side (fig. 1). The region of wound cork is usually somewhat swollen, increasing considerably the thickness of the leaf in the cicatrice region behind the pseudocicatrice. The preliminary barrier

is not displaced, however, as it is fixed in position by the overarching epidermal layers.

As early as the fifth day the cicatrice region shows positive reaction to phloroglycin tests for lignin, and during the following week this reaction becomes more marked. By the twentieth day the lignin seems to be restricted to a narrow zone outside the cicatrice proper. From the twentieth day on, all of the outer portions of the cicatrice stain with Sudan III, showing that this tissue becomes suberized as it matures.

II. Gaultheria shallon

In the region of study, San Juan Island in Puget Sound, *Gaultheria shallon* is usually a low shrub. Abundant in semiopen areas, it thrives with partial shade as a bushy undergrowth, but is often quite tall along forest borders. The large oval leaves are evergreen, about 250 μ in thickness, and usually live from two to four years (1). Transverse sections show a simple epidermis above and below, with about six interior layers of mesophyll cells (fig. 2). In addition to a single compact layer of short palisade next to the upper epidermis, there are one or two additional layers of isodiametric cells which are loosely arranged, leaving large intercellular spaces. The spongy mesophyll consisting of three or four layers has cells elongated and lobed in the horizontal plane, surrounding large air spaces. These tissues may be interpreted satisfactorily only from sections cut parallel with the epidermis. The veins are bordered above and below by heavy mechanical tissue, and the intervascular interval is 330 μ.

Wound responses

The exposed tissues along wounded margins collapse promptly but continue to die back slowly, so that it is several days before the pseudocicatrice takes its final form. The incurved cuticular layers may have completely folded over the wounded margin, unless prevented by adjacent supporting veins (fig. 2). By the tenth day, when cell enlargement begins in the region of the future cicatrice, there develops a sharp distinction between dead and living tissues. By the end of the second week several outer cell layers of the protected living tissue have enlarged until tightly pressed together, and their outermost walls have thickened considerably, but few cells have

as yet divided at this stage. In the spongy mesophyll the enlargement is most marked, with lobings and extensions of cells which bulge into the considerable spaces of this region. The developing cicatrice at this stage involves only 4–6-cell layers.

By the twentieth day cell enlargement in the cicatrice region has become marked and some mitoses may have taken place. The enlarging cells become highly distorted as they push past each other into the spaces of the mesophyll, and outward against the yielding pseudocicatrice. By the fortieth day the situation has completely changed (fig. 2). Repeated cell divisions have established a cicatrice 10–20 cells thick. The outermost, derived from certain of the cells that enlarged earlier, are much compressed and pushed outward by the pressure of those later formed behind. The non-suberized interior cells underneath the cicatrice proper enlarge rather more than is usual for this region.

The cicatrice of *Gaultheria shallon* requires over a month for its completion, and cell divisions are delayed longer than in any other form studied; mitoses may be retarded until three weeks after wounding. In many cases the cells of the cicatrice are telescoped, the inner extending lobes into the outer. This situation is made possible by the local thickening of walls on the outer side of cells, leaving inner walls relatively thin and subject to compression on that side. The pseudocicatrice is so firmly anchored by the epidermal layers that it is not displaced by this pressure from the growing tissues underneath. In all later stages the cicatrice region reacts positively to tests for both suberin and lignin.

III. Berberis nervosa

The Oregon grape, while not abundant in the region of the San Juan Islands, is rather common in partly shaded areas and along forest borders. Its stiff compound leaves, although about the same thickness as those previously discussed, are firm and usually persist for several years (1). Since the mature leaf of *B. nervosa* growing in this locality is unable to develop a pseudocicatrice following wounding, its foliar structure was studied with critical interest. Failure to develop this preliminary barrier inhibits in turn the development of a true cicatrice. Ultimately the air spaces in a zone of varying

width are blocked by modified tyloses. Fungal hyphae are usually abundant in the wounded margins, and masses of spores are seen at the edge of the dead tissue (fig. 4). These unusual wound responses are correlated with certain structural peculiarities that have been noted only in the leaves of this species. Transverse sections through

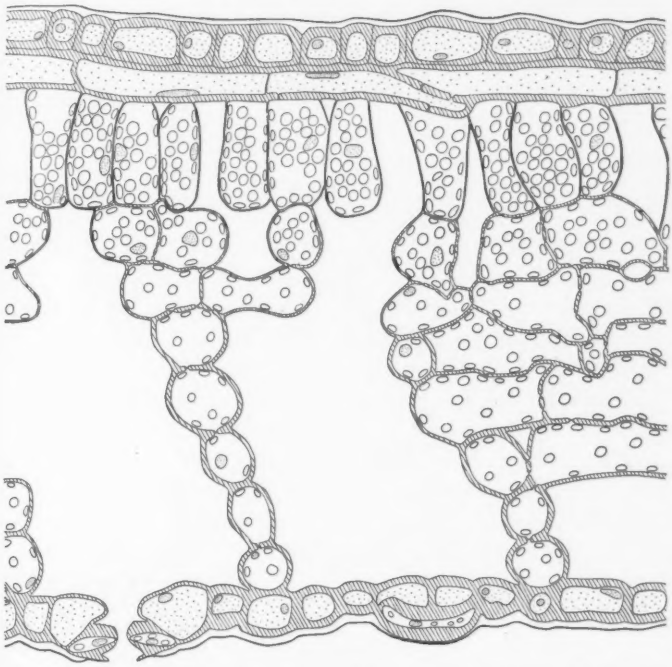


FIG. 3.—Transverse section of mature leaf of *Berberis nervosa*; heavy subdermal layer lies between upper epidermis and palisade; all cell walls much thickened.

the leaves show that this rigidity of structure is due primarily to the unusual thickness of all cell walls (fig. 3).

The sturdy epidermal layers, with heavy cuticular reinforcements, offer the usual specializations and have all stomata on the under side. There is also some cutinization of the inner surface of the lower epidermis, opposite the large intercellular spaces of the mesophyll. Next to the upper epidermis is a rigid subdermal layer of greatly

elongated thick-walled cells, which lie side by side in a compact layer continuous throughout the leaf area. A pinna dehydrated and cleared, thus permitting direct observation, showed that these cells are elongated parallel with the bases of the major veins, and continue this trend to the tips of the several lobes at the outer end of the leaf. This general direction is maintained in areas far removed from primary veins, and seems to be independent of the secondary venation. The cells of this mechanical layer are nearly square in cross-section, and are ten times as long as wide. The pitted walls are greatly thickened, and constitute a heavy structural reinforcement of the blade (figs. 3, 4).

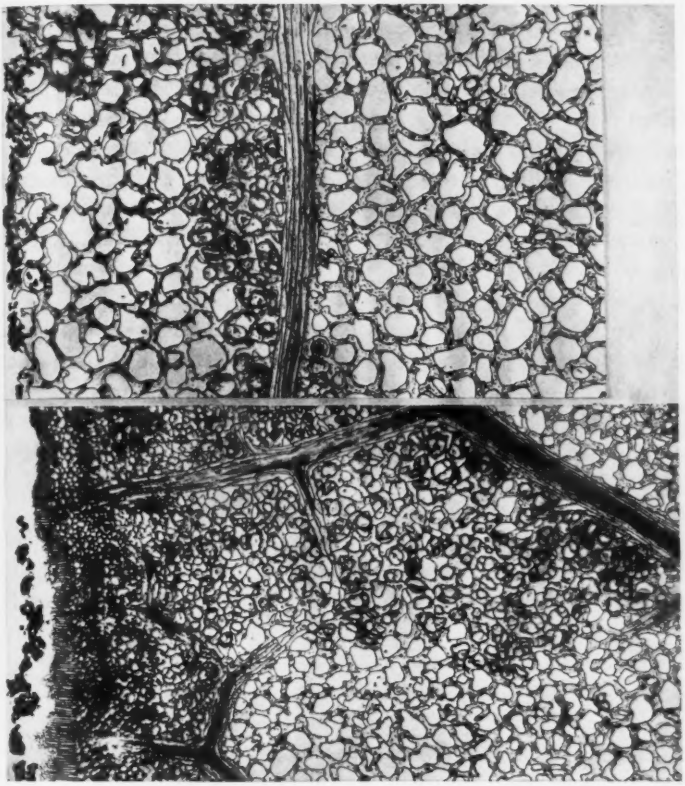
There is a single, simple palisade layer with cells about twice as long as wide, and underneath a layer of scattered squarish cells. This zone of the leaf has the thinnest walls and is especially rich in chloroplasts. The upper palisade layer, while rather compact, has spaces between its cells, and frequent larger openings lead up through it to the subdermal layer (fig. 3).

The five or six layers of spongy mesophyll have their cells elongated horizontally, and arranged in symmetrical meshes resembling the nets of *Hydrodictyon* but with a different cell arrangement (figs. 5, 6). These several layers have their meshes superimposed, surrounding relatively large air spaces, and appear in cross-section as columns of cells which tie the lower epidermis to the palisade region. This arrangement offers a degree of mechanical support not usually associated with the porous mesophyll. The intervascular distance is unusually great (392 μ); this wide space between veins is probably correlated with the reduced palisade and the horizontal elongation of mesophyll cells.

Wound responses

The combined structural features of the leaf of *Berberis nervosa* make it peculiarly rigid. Not only are all cell walls unusually thick, but the spongy mesophyll is so arranged as to offer almost the rigidity of solid tissue. There are in addition the heavy-walled epidermal layers, and the leaf is further fortified on the upper side by the strong subdermal mechanical cells already described. This combination of structural features results in a leaf, which if mature when wounded, cannot collapse following lesion. Although the lifeless protoplasts

are shrunken within their walls along the wounded margin, there is no crumpling of any portion of the leaf tissue, all parts retaining their



FIGS. 5, 6.—Fig. 5 (above), section of *Berberis nervosa* 40 days after wounding of mature leaf, cut parallel with epidermis and showing netlike arrangement of spongy mesophyll; dead tissue at left has fungal hyphae and spores, and zone of tyloses borders large vein on left side; normal leaf tissue at right. Fig. 6 (below), section of leaf of *Berberis nervosa* 40 days after wounding of mature leaf, cut parallel with epidermis, dead tissue along margin is darkened by masses of fungal spores, and tyloses have extended far into leaf in region without larger vein.

original relations (fig. 4). Consequently there can be no formation of pseudocicatrice. In the absence of such protective barrier, the many

air spaces of the mesophyll remain wide open, although separated somewhat by the ranked and meshed arrangement of the cells. The exposed marginal tissues die back irregularly, usually to the region of the first important vein. This results in a dead zone of varying width (figs. 5, 6), in striking contrast to the usual rather uniform margin of lifeless tissue along the wounded edge. These dead mesophyll cells often contain considerable starch, which encourages the growth of certain types of fungi (figs. 4–6). Sections of living leaves show that the mesophyll is commonly used in summer for storage of starch, this being one reason for the relatively dull appearance of their under surface. In the sister species, *B. aquifolia*, the zone of dead tissue is nearly uniform in width, and the region of the well developed cicatrice runs parallel with the marginal pseudocicatrice.

It is of special interest that under the circumstances just discussed, which prevent the formation of a pseudocicatrice, there is no true cicatrice developed if leaves of *B. nervosa* are mature when wounded. Instead of a localized zone of wound cork, the intercellular spaces in a region of varying width behind the dead tissues are ultimately blocked by a system of tyloses formed from adjacent mesophyll cells (figs. 4–6).

Growth changes take place slowly in this species. By the fifth day there is a faint suggestion of lignin in the outer margin of living cells. On the tenth day, when cell enlargement is evident, the walls of this region react to tests for both lignin and suberin, the latter being much more pronounced nearer the epidermal layers. Both of these secretions persist in the region of wound response, even where there is little change in size and form of cells.

The tissue changes involved are limited practically to cell enlargement, first noted in the outer portion of living cells about the tenth day. Mitoses seem rarely to occur, although cells may continue to enlarge for three weeks. The width of the zone undergoing such modification varies greatly, and is related in a general way to venation. If a larger vein is near the wounded edge, its outer side is bordered by a narrow zone of greatly enlarged cells (fig. 5); on the other hand, if no important vein is near the wounded edge, there may be a wide region of marked cell enlargement (fig. 6).

The most striking developments are in the spongy mesophyll,

which is the most extensive of the interior tissues of these leaves. Living cells surrounding the meshes enlarge into the extensive intercellular spaces. Viewed in sections parallel with the epidermis (figs. 5, 6), it may be noted that the enlargements into spaces may be numerous; two or more may meet and flatten together like tyloses in the tracheal vessels of stems. These intrusive swellings, or tyloses, may be very large, amounting to many times the volume of the original cell of which each remains a part. Ultimately over a consid-

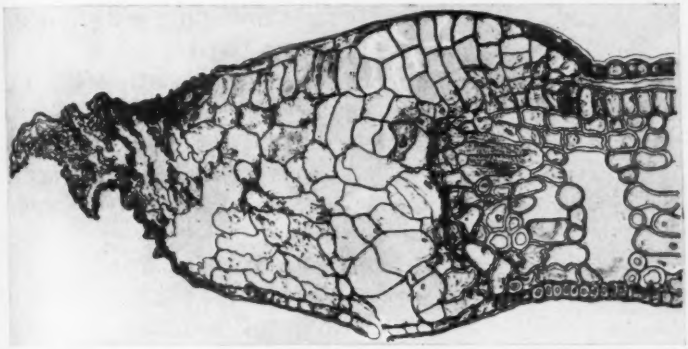


FIG. 7.—Transverse section through younger leaf of *Berberis nervosa* wounded before maturity; pseudocicatrice very compact and cicatrice involving massive development of wound cork which in some cases ruptures epidermis; normal leaf tissue at right of figure; killed 40 days after wounding.

erable width of tissue the air spaces are occluded. There is no suggestion of wound cork in these leaves under such circumstances.

WOUND RESPONSES OF YOUNGER LEAVES

Among the leaves of *B. nervosa*, wounded and collected for the writer by Dr. GEORGE B. RIGG of the University of Washington, was one with well developed cicatrice along all wounded margins (fig. 7). Recalling that the wound responses of older and younger leaves may vary considerably, the writer assumed, and apparently correctly, that the leaf thus forming a cicatrice had been wounded earlier in its development. The following year Dr. RIGG kindly carried out further wounding experiments and intentionally included younger leaves which had developed that spring. Among these, when pre-

served and shipped to our laboratory, were found many that had formed the pseudocicatrice, and this in turn had been followed by a cicatrice sometimes of striking development.

In fig. 7 are shown the wound responses of an immature leaf wounded on May 21 and killed June 30, 1927. It may be noted that there had been complete collapse of the mesophyll following lesion. Not only had the interior tissues crumpled, but both epidermal layers were drawn sharply inward, the leaf shrinking to less than one-third its original thickness and giving rise in this way to a most efficient pseudocicatrice. It was significant to find that under such circumstances a cicatrice also was developed, and that wound cork was formed in abundance. In most instances the cicatrice region was swollen to nearly double the normal leaf thickness, and in some cases the growth of wound cork even burst the sturdy epidermis on the under side of the leaf. All cell layers contributed to this riot of wound tissue development, and the heavy subdermal layer was displaced through a considerable distance (fig. 7).

A study of leaves of *Berberis nervosa* wounded at varying ages shows a close correlation between the degree of pseudocicatrice development and the amount of cicatrice formation. Intermediate stages, in which the dead leaf tissues along the wound margins collapse to about one-half the normal leaf thickness, show considerable activity of living cells along the upper part of the leaf. In such cases, behind the partial pseudocicatrice, mitoses in the palisade cells produce two, three, or occasionally four layers of cork extending through a considerable distance along the upper epidermis, and resulting in a decided bulge on this surface. The lower portion of the leaf, however, responds by the formation of tyloses, and these are restricted to a relatively narrow zone. Fungal mycelia are but sparingly developed in such cases.

In those instances where the leaves were younger and dead tissues collapsed in greater degree, approaching the normal pseudocicatrice, cork formation involved all layers of the leaf, and thus provided a symmetrical healing tissue of wound cork launched evidently through mitoses in all cell layers. In this species there is therefore a diminishing capacity for wound response as the leaf matures, and all intermediate stages may be secured between the extremes shown in figs. 4 and 7.

Practically every section through any leaf of *Berberis nervosa*, if mature when wounded, shows numerous fungal hyphae and spores in the dead marginal tissue. The most conspicuous fungus is a species of *Fumago*, as determined by Professor G. W. MARTIN, and the sclerotia of this form may literally blacken the wounded leaf margin (figs. 4–6). The growth of this particular fungus may have been favored directly or indirectly by stored starch left in the dead cells, which in the absence of a pseudocicatrice, were freely exposed to fungal invasion. No such growths were noted in the leaves of this species if wounded at an earlier stage when both cicatrice and pseudocicatrice could be developed (fig. 7).

Mycelia of fungi have been noted rarely in studying the wounded leaves. Among some hundreds of species examined, many of them natural wounds, the only other ones regularly harboring living organisms were certain thick-leaved New Zealand evergreens which had yeastlike cells in the pseudocicatrice. Lodged spores have often been noted, but active mycelia seem rare. This is in harmony with the general observation that exposed foliage leaves, although subjected to constant injury, are seldom infected through open wounds. Even spores carried on the biting parts of insects may be excluded by the barriers subsequently developed, for the dying and drying of exposed leaf tissue, as the pseudocicatrice forms, may inhibit spore germination or mycelial growth. Meanwhile the whole marginal region is soon walled off by the tissues of the cicatrice proper. Of course when the spore or inoculum is introduced into the tissues by a suctorial insect such protective devices do not function. Following lesions of this type, neither pseudocicatrice nor cicatrice is formed, and infection if it occurs is underneath the normal leaf coverings.

Discussion

The broad-leaved evergreens discussed in this paper offer numerous contrasts to the mesophytic types previously described (3). Their leaves are more massive than those of the deciduous forms, cuticular coverings are much heavier, and all cell walls are thicker, especially in *Berberis nervosa*. In addition to these structural qualities there are probably other differences, doubtless of no less significance, relating to the content and modified activities of the cells. Wound responses, however, with the exception of *B. nervosa*, are

similar in a general way to those exhibited by mesophytic foliage, although the process is much slower and wound cork is usually produced much more abundantly.

These generalizations are not based alone upon the three species discussed in this paper. Unpublished data relate also to the broad-leaved evergreens from southern California, the strand vegetation of the Fiji Islands, and the preponderantly evergreen flora of North Island, New Zealand. In the considerable number of species examined from these widely separated regions, lesion of exposed leaves regularly results in the establishment of a pseudocicatrice through the collapse of dead tissues. This in turn is followed by the development of a more or less extensive cicatrice through the activity of living cells.

Recalling the more massive structure and heavier cell walls of such leaves, many of them sclerophylls, one might not have anticipated the regular formation of this preliminary barrier. While its initiation begins promptly, the period through which drying and dying back continues may be considerably extended, owing to differences in cell structure and content. The pseudocicatrice finally produced, however, is usually much more extensive than in the thinner and softer leaves; and the heavy cuticular layers, which are commonly involved, add much to its efficiency.

Comparable differences are noted in the cicatrice proper. Its beginning in these leaves is considerably delayed, and complete development requires much longer than in mesophytic leaves. The initial mitoses may not take place until ten days after lesion, and complete development requires a month or more. While the cicatrice is commonly more massive than in the mesophytes, owing to its tardy development there is a much longer period of dependence upon the pseudocicatrice for protection against traumatic water loss and possible infection.

The single exception encountered in this general survey of evergreen angiosperms was *Berberis nervosa*. With walls of its mature leaves too strong to collapse following the death of protoplasts, no pseudocicatrice can result; unique also is this form in its inability to develop wound cork under these conditions, and doubtless the common presence of fungi in the wounded margins may be correlated

with the lack of the usual protective barriers. Of no less interest is the strikingly different behavior of the younger leaves of this species. If wounded before the cell walls have fully thickened, an efficient pseudocicatrice is followed by an unusual development of wound cork. Leaves wounded at varying stages of maturity show different degrees of tissue collapse, and there is likewise a close correlation between the amount of pseudocicatrice development and that of wound cork formation. In fact all types of response might be brought about by wounding the leaves of a single plant at different stages of foliar development. It probably is not accidental that the growth of fungi in the wounded margin is inversely proportional to the development of these barriers.

It is unfortunate that a mature foliage leaf cannot regenerate the epidermal and cuticular layers when wounded. A cuticle less than 1 μ in thickness seems to meet admirably the problems of the average leaf's protection, but if wounded, the traumatic responses usually involve a wide zone 50–150 cells in depth, in healing the lesion. While very young leaves are reported to regenerate the epidermis when injured, older ones seldom do this even indirectly. Apart from the greater plasticity of younger leaves, the older foliage organs are necessarily in exposed position, and marginal cells abruptly laid bare by wounds cannot meet the problems of excessive water loss and maintain turgor long enough to develop a cuticular covering.

The cicatrice formation of a leaf is in principle like the development of healing tissues in stems, and especially in tubers. There is the establishment of a preliminary phellogen layer, and the subsequent development, by means of its activities, of a mass of wound cork. All of this takes considerable time, and in the case of leaves, gives rise to a healing tissue actually far in excess of the protective needs of the organ. The cicatrice tissues developed by a wounded leaf are therefore not peculiarly foliar specializations, but are typical of massive plant organs. On the other hand, the pseudocicatrice is distinctively a foliar response. Its favorable development is contingent upon cell structure, tissue arrangement, and organ form not usually found in stems or other massive plant parts. The fortunate combination in foliage leaves of a thin, flattened form, with soft interior mesophyll having yielding epidermal layers covered by a thin

elastic cuticle, provides for ready collapse of cells exposed by wounds, and results in a marginal protective barrier produced by these dead tissues. Perhaps without this pseudocicatrice the highly specialized foliage leaf, subject to constant injury, would have been impossible.

It remains for experimental work to interpret the causal relations between pseudocicatrice and cicatrice proper; there is considerable evidence that the latter is not only sequential to the other in time, but is likewise dependent upon it for development. Apart from such considerations, however, the pseudocicatrice is of primary importance as a protective device, and plays an even more important part in broad-leaved evergreens than in mesophytes.

Summary

1. Leaves, as necessarily exposed organs, do not employ massive coverings, and so are constantly subjected to injury.

2. Following lesion, tissue collapse along wounded leaf margins usually leads to the prompt establishment of a protective barrier, the pseudocicatrice.

3. The cicatrice proper of wound cork forms slowly underneath, from living tissues protected by the pseudocicatrice.

4. The broad-leaved evergreens in comparison with mesophytic foliage have thicker leaves, heavier cell walls, and better developed cuticular layers. The wound responses are similar, but the process is slower in the thicker leaves and their protective structures involve much greater amounts of tissue.

5. The mature leaves of *Berberis nervosa* are unique because they are so rigid that the exposed tissues cannot collapse; consequently these leaves are unable to form a pseudocicatrice, and this in turn inhibits the development of any cicatrice. Such leaves are left open to fungal invasion and usually have masses of spores and mycelia in and along the wounded margins.

6. If younger leaves of the same plants are wounded, the dead tissues collapse into an efficient pseudocicatrice, while behind this is formed a massive cicatrice of wound cork, and such protected margins have no fungi. These leaves reveal a diminishing capacity for the formation of such protective barriers as they approach maturity, and show increasing amounts of fungi along their wounded margins.

7. The cicatrice proper is generally not initiated until several days after injury, and its complete development in broad-leaved evergreens requires 20–40 days. While highly efficient, it is usually more elaborate than necessary, and corresponds in many respects with the healing tissues of tubers or other massive plant parts.

8. The pseudocicatrice is distinctively a foliar response. The structure and arrangement of cells in the leaf in combination with its thin and flattened form permit the ready collapse of tissues exposed by wounds, with favorable use of the cuticular layers. The pseudocicatrice, in addition to its protective value, sustains a causal relation to the development of wound cork and remains a permanent barrier along the wounded leaf margin.

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RELATIVE ABUNDANCE OF STOMATA IN CITRUS AND SOME RELATED GENERA[1]

E. HIRANO[2]

(WITH ONE FIGURE)

Citrus trees are cultivated in tropical and subtropical regions under a variety of climatic and soil conditions. In semiarid regions there are often deficits of water which induce a suspension or temporary modification of fundamental physiological processes. Fluctuations in the water content of citrus leaves were reported by COIT and HODGSON (3), who pointed out the effects of the deficits upon the abscission of young fruits. BARTHOLOMEW (2) described the effects of daily deficits of water in the lemon tree, and REED and BARTHOLOMEW (6) studied the effects on orange trees of more prolonged water deficits. OPPENHEIM (5) described the diurnal changes in the activity of stomata and made determinations on their density in *Citrus aurantium* and *C. aurantifolia*.

The writer studied the relative abundance of stomata in the leaves of various species of *Citrus* and related genera, with special reference to their geographical origin, so far as it could be ascertained. The material for this study was collected in a restricted area, however, namely, the orchard of the Citrus Experiment Station at Riverside, California. The study may therefore be expected to indicate whether there were any heritable differences in the abundance of stomata when all the species and varieties were grown simultaneously under the same climatic and geographic conditions.

I am indebted to Dr. H. J. WEBBER for freedom to use the necessary material, and to Dr. H. S. REED for the facilities of the laboratory of plant physiology, as well as for his advice and assistance.

The leaves used for this study were collected during a period ex-

[1] Paper no. 244, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] Professor HIRANO spent most of the year 1929 as a guest investigator in the Citrus Experiment Station. This manuscript contains some of the results which he obtained during that time (H. S. R.).

tending from September 16 to 30, 1929, from shoots produced during the first cycle of growth of that season. Care was taken to select full-grown leaves from non-fruiting shoots which grew in full sunlight on the south side of the tree. For each species or variety a sample of 15 to 20 leaves of similar area from as many shoots was selected. Particular attention was paid to the selection of leaves of approximately the same size and age, since it has been found that these factors influence the density of stomata (REED and HIRANO 7).

TABLE I

CLIMATOLOGICAL DATA FOR 1929 AT RIVERSIDE, CALIFORNIA; LATITUDE 34° N.; ELEVATION ABOVE SEA LEVEL, 851 FEET (262 M.)

	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
Precipitation (mm.)	35.3	21.1	23.4	64.5	Trace	Trace	0	0	16.0	0	0	0
Mean temperature (° F.)	49.6	49.2	54.8	56.2	65.2	69.7	76.2	79.0	70.8	67.0	58.4	60.6
Sunshine*												
Actual number of hours	209	236	275	259	300	350	352	374	265	283	299	246
Per cent of possible	66	77	74	66	69	81	80	90	71	81	96	80
Relative humidity (percentage)	79	67	66	73	68	60	65	53	80	61	42	63
Evaporation from pan (inches)	2.03	20.5	3.25	4.92	6.0	7.86	11.39	8.77	7.23	4.93	5.56	3.97

* Data on sunshine taken from records obtained by Weather Bureau in Los Angeles.

After measuring the leaf areas with a planimeter, 10 similar leaves were selected from each species or variety and small pieces of tissue cut from the middle of each leaf. These pieces were kept in acetic-formalin-alcohol until the stomata were counted.

The number of stomata in 20 areas of 0.29 sq. mm. was determined for each leaf, and from this determination the average number per square millimeter was computed. The number of stomata in 200 distinct areas for each species or variety employed was therefore determined. Areas in the vicinity of large veins, oil glands, and trichomes were avoided, since they contain relatively few stomata.

The nature of the climatic conditions prevailing at Riverside may be inferred from the brief summary given in table I. The data show that the rain of the year fell principally in the first four months, and that evaporation was high in the seven months following April. To

be more exact, more than half the precipitation occurred in the months of March and April, a period in which many of the new leaves were growing. The importance of temperature and precipitation in the months of March, April, and May is indicated by data obtained by BAHGAT (1) and summarized in table II. It is impossible, of course, to dissociate the effects of the two factors in the present case, but it appears probable that the abundance of stomata shows a closer correlation with precipitation than with temperature.

BAHGAT (1) found that (1) density of stomata on the leaves of a given species or variety of *Citrus* was greatest in hot arid regions

TABLE II

RELATION OF NUMBER OF STOMATA TO AMOUNT OF PRECIPITATION AND TEMPERATURE IN VARIOUS LOCALITIES IN CALIFORNIA (DATA ON STOMATA TAKEN FROM BAHGAT)

LOCALITY	LATITUDE (N.)	MEAN TEMPERATURE		PRECIPITATION		NO. STOMATA PER SQ. MM.						
		March to June (° F.)	Year of 1922 (° F.)	March to June (mm.)	Year of 1922 (mm.)	Washington Navel	Sour orange	Grapefruit	Eureka lemon	Valencia	Mandarin	Dancy tangerine
El Centro*...	32°51′	73.0	71.0	Trace	65.3	550	498	617	555	465	.....	.....
Riverside....	34° 0′	62.8	62.8	77.2	343.9	468	434	479	530	465	420	466
Porterville...	36° 0′	65.8	64.4	104.1	320.9	348	390	463	494	372	382	390
Berkeley.....	37°54′	56.5	56.0	111.3	764.54	358	364	399	480	.....	363	376

* Data recorded at Calexico near El Centro.

and least in cool moist regions; (2) shading the leaves reduced the density of stomata and increased their size; (3) leaves of the grapefruit and lemon had a greater density per unit area than other varieties studied, while a smaller number was found on leaves of the kumquat and trifoliate orange.

In presenting the following report, the precipitation for the spring months (March 1 to June 1) is recorded in addition to the amount of annual precipitation. When dealing with places in the southern hemisphere, data for the corresponding season (September 1 to December 1) are used. Most of the data on rainfall in tables III and IV were obtained from standard works on meteorology, among which FISCHER (4) should be noted.

The ecological relations of cultivated citrus trees are difficult to define, because the cultivator employs irrigation of the soil to avoid

TABLE III

DENSITY OF STOMATA ON LEAVES OF VARIOUS SPECIES OF CITRUS GROWN AT RIVERSIDE, CALIFORNIA, IN 1929

C. E. S. RECORD NO.	SPECIES OR VARIETIES	AVERAGE LEAF AREA (SQ. CM.)	AVERAGE NO. STOMATA PER SQ. MM.	NATIVE PLACE OR MAIN LOCALITIES OF CULTIVATION	PRECIPITATION		LOCATION OF STATION
					Spring mos. (mm.)	Annual (mm.)	
628	C. aurantium Standard Seedling	36.0	414±2.3	Western Spain; domesticated in Florida	87	438	Seville, Spain
					495	1525	Miami, Fla.
					431	1374	Bartow, Fla.
					225	787	Caetite, Bahia, Brazil
1110	Brazilian	22.9	440±2.5	Brazil	481	1910	So. Bento das Lager, Bahia, Brazil
					382	1091	Rio de Janeiro, Brazil
660	Paraguayan	21.7	459±2.7	Wild in Paraguay	380	1423	Asuncion, Paraguay
					509	1465	San Salvador, Paraguay
1226	Chinese "Tsen Tze"	40.2	465±3.0	Ichang, China; wild in Himalaya	428	1059	Ichang, China
					284	1024	Yunnanfou, China
					140	955	Allahabad, Punjab
					445	1055	Durban, Natal
64	South African	23.6	490±2.1	So. Africa	175	550	Bloemfontein, Orange Free State
					322	746	Pretoria, Transvaal
564	C. aurantifolia Bearse Seedless lime	13.2	326±2.4	Originated in California	118	440	Los Angeles, Calif.
391	Tahiti lime	14.2	375±3.2	Tahiti Island	483	1410	Papeete, Tahiti
					431	1373	Bartow
452	Kusai lime	14.5	595±2.8	Kusai Island; important lime for Hawaii (Hume)	632	1760	Lalahafen, Kusiae
					210	755	Honolulu
					665	2100	Av. of all Hawaiian Island
1710	Mexican lime	13.6	514±2.6	Hawaii; wild in forest of So. Florida	210	755	Honolulu
					495	1524	Miami
1214	C. cambrovioxora (probably C. hystrix)	10.6	384±2.9	Tahiti	482	1410	Papeete
1665-B	C. junos Yuzu	12.8	429±2.3	Wild in Yunnan; cultivated in Japan, Korea	284	1024	Yunnanfou
					555	1476	Tokyo, Japan*
1216	Kansu†	12.2	402±2.4	Hupeh, China; cultivated in Japan	555	1476	Tokyo*
432	C. hystrix Large leaves and large smooth fruit	28.0	550±2.4	Islands of tropical Pacific, Indian oceans	381	1962	Manila, P.I.
					871	2190	Colombo, Ceylon
	Small leaves and small corrugated fruits	12.8	704±3.5	.....	.....	.....	.....

TABLE III—*Continued*

C. E. S. RECORD NO.	SPECIES OR VARIETIES	AVERAGE LEAF AREA (SQ. CM.)	AVERAGE NO. STOMATA PER SQ. MM.	NATIVE PLACE OR MAIN LOCALITIES OF CULTIVATION	PRECIPITATION		LOCATION OF STATION
					Spring mos. (mm.)	Annual (mm.)	
	C. limonia						
400	Rough lemon	39.3	552±2.8	Naturalized in forests of So. Fla.	495 431	1525 1374	Miami Bartow
1482	Sweet lemon	39.1	635±2.5	Cultivated in Mediterranean regions	137 116	590 362	Palermo, Italy Murcia, Spain
	Eureka lemon	37.0	636±2.5	Originated in Los Angeles	118	400	Los Angeles
584	Lisbon lemon	40.5	743±3.6	Mediterranean and California	116 137 118	362 590 400	Murcia Palermo Los Angeles
	C. maxima						
1462	Cuban shaddock	44.8	476±3.7	Cuba	427 507 337	1794 1396 1092	Union de Reyes, Cuba Average of 19 observatories in Cuba Havana, Cuba
1852	C. nobilis Kunembo	37.6	377±4.8	Liu-kiu Island, Japan	552	1476	Tokyo
277	Dancy	12.1	415±1.8	Florida and Foochow, China	? 431	1515 1374	Foochow, China Bartow
	Unshiu (Satsuma)	40.6	446±2.8	Southern Japan, Florida, Alabama, etc.	431 552	1374 1476	Bartow Tokyo
594	King	13.3	463±2.7	Cochin-China, Florida, China, Japan and Luchu	431	1374	Bartow
270	Cleopatra	11.8	552±3.7	Florida and Algiers	431 196	1374 546	Bartow Constantine, Algiers
696	Kinokuni	12.7	582±2.6	Kiangsi and Chekiang; imported from Ichang, China	428	1059	Ichang
	C. paradisi						
325	Chinese pomelo	42.9	532±3.8	.....	431 495	1374 1525	Bartow Miami
577	Shaddock (Calcutta)	40.8	571±4.0	Calcutta	534	1652	Calcutta
454	Shaddock (Hawaii)	40.7	626±3.0	Honolulu	211	756	Honolulu
579	Ditto	44.5	630±3.1	Vicinity of Kilauea	211	756	Honolulu
	C. medica						
138-B	Indian citron	21.1	552±2.2	Seeds from India	534	1652	Calcutta
757	Phillipine citron	32.8	623±3.3	Seeds from Lamao Exp. Sta. Batan, P.I.	382	1962	Manila
601	Chinese lemon	22.5	643±3.3	Mongtze, Yunnanfou, China	317	955	Mongtze, China
128	Italian citron	19.6	873±4.7	Buds from Posilipo	137	590	Palermo

TABLE III—*Continued*

C.E.S. RECORD NO.	SPECIES OR VARIETIES	AVERAGE LEAF AREA (SQ. CM.)	AVERAGE NO. STOMATA PER SQ. MM.	NATIVE PLACE OR MAIN LOCALITIES OF CULTIVATION	PRECIPITATION		LOCATION OF STATION
					Spring mos. (mm.)	Annual (mm.)	
1458	C. mitis.....	13.4	665±7.7	Endemic in Philippines and interior of China	382	1963	Manila
	Marsh.....	42.4	583±3.2	West Indies; cultivated in Florida and California	117	400	Los Angeles
					431	1373	Bartow
					495	1525	Miami
448	Round Hawaiian pomelo..	41.0	584±2.6	Honolulu	210	756	Honolulu
	C. sinensis						
255	Lue Gim Gong orange.	40.8	402±2.5	Florida	431	1373	Bartow
					495	1525	Miami
	Washington Navel orange	41.1	458±2.7	Originated in Brazil; cultivated mainly in California, So. America, Australia, So. Africa	225	787	Caetiti, Bahia
					117	400	Los Angeles
					322	746	Pretoria
282	Jaffa orange...	38.8	473±1.8	Mediterranean	266	947	Beirut, Syria
	Valencia orange (large leaf)...	38.7	504±2.7	California and Mediterranean	113	476	Valencia
					117	400	Los Angeles
591	St. Michael orange (paper rind).....	41.2	531±2.7	Mediterranean	107	608	Malta
					130	430	Alicante, Spain
					167	976	San Miguel, Azores
209	Ruby blood orange.....	36.1	533±2.5	Mediterranean	183	771	Phillippeville, Algiers
					113	476	Valencia
1455	C. webberi	9.5	769±6.0	Endemic in Philippines	382	1963	Manila

* Precipitation at Tokyo is not representative of precipitation in Japanese orange regions, but since data for orange regions is not at hand, the precipitation of Tokyo is used here.

† Relationships of Kansu not yet determined.

drought, heating devices to maintain temperatures above the freezing point, and other means for making a suitable environment. Nevertheless cultivators have found by the trial and error method that certain varieties may be grown successfully in certain localities but not in others.

It seems evident from the data in tables III and IV that species whose leaves have an average density of 500 or more stomata per square millimeter thrive in the tropics, with the exception of lemon

TABLE IV

DENSITY OF STOMATA ON LEAVES OF VARIOUS SPECIES OF AURANTIOIDEAE GROWN AT RIVERSIDE, CALIFORNIA, IN 1929

C. E. S. RECORD NO.	SPECIES OR VARIETIES	AVERAGE LEAF AREA (SQ. CM.)	AVERAGE NO. STOMATA PER SQ. MM. (20 COUNTS TO LEAF)	NATIVE PLACE OR MAIN LOCALITIES OF CULTIVATION	PRECIPITATION		LOCATION OF STATION
					Spring mos. (mm.)	Annual (mm.)	
1465	Eremocitrus glauca	0.6	167±1.7	Desert in northeastern Australia	?	?	.....
1484	Microcitrus australasica	0.4	407±2.5	Moreton Bay, near Brisbane, Queensland	301	1265	Sydney, Australia
1466	Faustrimedin (C. mitis ×M. australasica)	1.6	409±2.0	.....	.....	.....	.....
268	Fortunella margarita	8.6	461±3.3	Japan	552	1476	Tokyo, Japan
1484	Microcitrus australasica var. sanguinea	0.4	485±2.2	Tamborine Mountain, So. Queensland	301	1265	Sydney
	Poncirus trifoliata	5.0	495±3.8	Western Hupeh; Ichang, Shensi, China; Japan and Korea	428	1059	Ichang, China
					552	1476	Tokyo
1637	Chalcas exotica	10.6	501±2.2	Indian peninsula, India, Ceylon, Burma, Indo-China, Malay, China, Japan, etc.	534	1652	Calcutta, India
					871	2190	Colombo, Ceylon
1433	Balsamocitrus paniculata	6.7	543±2.7	Tropical W. Africa (Nigeria, Gold Coast)	.....	2000–3000	Nigeria; most parts of Gold Coast
1432	B. gabonensis	12.2	553±2.3	Pahouin No. French Congo, So. Kamerun	.....	2000–3000	Kamerun-No. French Congo
1450	Citropsis schweinfurthi	10.4	589±3.5	Uganda, Congo, Sudan	.....	2000–3000	Congo, Uganda, most parts of Sudan
1471	Chaetospermum glutinosum	8.6	592±3.0	Central part of Luzon	382	1963	Manila, Luzon, P.I.
1430	Atalantia citroides	9.1	697±2.5	Cochin-China, Cambodia	600	1956	Saigon, Cochin-China Khong, Cambodia
					755	2159	
1638	Atalantia disticha (blanco)	2.9	867±4.8	Philippines	382	1963	Manila, Luzon, P.I.
1492	Severinia buxifolia	3.5	1064±3.7	So. China	327	2795	Hue, Annan
				Hongkong, Kwantung	548	1614	Tonkin
				Hainan, Tonkin	526	1631	Hoihow, Hainan, China
				Annan, Formosa, etc.	737	2005	Hongkong, China

and grapefruit. It should be noted, however, that the lemon is probably a native of India, although it is extensively cultivated in Sicily and California. Species which have a stomatal density less than 500 are generally found in regions between 23.5 and 44 degrees of latitude north or south of the equator, with the exception of *Citrus aurantifolia* and *C. cambrovioxora*. A variability of less than 100 stomata per sq. mm was observed among the varieties of *C. aurantium*, *C. junos*, and *C. paradisi;* of 100–200 stomata per sq. mm. in *C. hystrix*, *C. limonia*, *C. maxima*, and *C. sinensis;* while a variability of more than 200 stomata was found among varieties of *C. aurantifolia*, *C. medica*, and *C. nobilis*. The abundance of stomata in the leaves of certain other genera of Aurantioideae was also investigated, so far as material was available in the orchard of the Citrus Experiment Station. The results (table IV) will be presented before proceeding to discuss the more general problem.

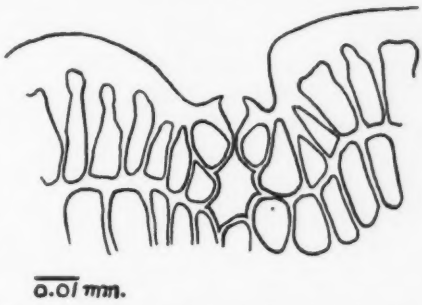

FIG. 1.—*Eremocitrus glauca*, section through stoma.

The species listed in table IV are not cultivated to any great extent, and it is therefore interesting to compare their characters, because it may be assumed that they have been but little changed by artificial selection or by long cultivation outside their habitats. The density per square millimeter varied from 167 in the leaves of *Eremocitrus glauca* to 1064 in the leaves of *Severinia buxifolia*. *E. glauca* is a native of the Australian deserts, and has characters like those commonly found in desert plants, small pubescent leaves, stomata imbedded in the body of the leaf (fig. 1), etc. The number of stomata per square mm. was very small. *Microcitrus australasica* and its variety *sanguinea* also have very small leaflets, but the density of stomata is much greater than that of *E. glauca*. The leaves of *Faustrimedin*, a hybrid, resemble more closely in the matter of size and density of stomata those of *M. australasica* than any other parent.

It will be noted that the species which had a stomatal density less than 500 occur generally in subtropical and temperate zones, and that species having a density greater than 500 occur generally in the tropical zone, although *S. buxifolia* and *C. exotica* have a wide range and extend somewhat into the subtropics. The number of species observed is not large, and one must be cautious about drawing rigid conclusions from them. However, they indicate strongly that the species and varieties of tropical regions studied have a greater stomatal density than those of extra-tropical regions. I hope to study this problem further. The two species of *Atalantia* had a relatively high stomatal density, but *Severinia buxifolia* was considerably higher than any other species studied.

In considering the relative abundance of stomata in the species and varieties listed in tables III and IV, the discussion will deal incidentally with the potentiality of the environmental factors which affect their abundance on the leaves of *Citrus* and related plants. The number of stomata per unit area in various plants has been found by previous investigators to be influenced by light intensity, water content of soil, and atmospheric humidity. The effect of air temperature has not been studied sufficiently to warrant any positive statements about it. High temperatures are usually associated with strong light intensity and low humidity. Table II indicates that precipitation during the period in which leaves are developing is more important than temperature. For example, the differences between the numbers of stomata in various species at Porterville and Berkeley are not significant, but the differences in precipitation in the spring months is likewise small.

The intensity of insolation in the tropics is greater than in other regions of the earth, and this is associated with a higher mean temperature. Both insolation and temperature may be reduced locally by precipitation and attendant cloudiness, although the mean cloudiness in various latitudes does not vary as much as might be expected. The ensemble of the factors and not individual factors needs to be borne in mind when considering the generic and specific differences in leaf size and stomatal density presented in tables III and IV.

Between latitudes 23.5° N. and 40° N. there are two distinct cli-

matic conditions with respect to citrus culture; namely, Mediterranean climate and Temperate climate. The Mediterranean climate mostly predominates in the citrus districts along the Mediterranean Sea and the southwestern part of the United States (southern California and Arizona). In these districts the annual precipitation is small, and most of the rainfall comes in winter as intermittent storms separated by spells of fine sunny weather. Although the natural intensity of light in these regions may be inferior to that of the tropics, therefore, the absorption of light by clouds, rains, etc., is low and the moisture in air and soil is very small. Such conditions may be more favorable for the development of high stomatal density, or for the adaptation of *Citrus* varieties having high density, than other districts of similar latitude having moist climates. These districts contain many famous commercial orchards including varieties of comparatively high density; that is, various kinds of lemons, sweet oranges, and grapefruits. Even in these districts the distribution of species is governed by slight changes in climatological factors. The general classification of varieties of *Citrus* of subtropical regions of southwestern United States having the so-called Mediterranean climate will give some idea of the relations between stomatal density and adaptability to climatic conditions (table V).

The Navel orange, which has the lowest density of stomata among the typical varieties, is grown in the northern part of this area, which is characterized by light to moderate rainfall, hot dry summers, and cold winters. The Valencia orange has a slightly higher density of stomata than the Navel orange, and is grown somewhat farther south and nearer the sea coast, where there is rather more rainfall in the winter and higher humidity in the summer. The grapefruit, which has a higher density of stomata than the Valencia orange, is grown in the more arid regions of the southwest. It seems that the climatic factors associated with precipitation and intensity of light have an important influence on the geographical distribution of these three varieties.

The citrus districts having the so-called Temperate climate appear to have an annual precipitation which is a little less than that of the tropics. Although precipitation is usually much greater in the summer growing season than in the winter resting season, there are

no such distinct seasons of extreme dryness and wetness as in the so-called Mediterranean climate or in the tropics.

The main citrus districts in the Temperate climate seem to be poorly suited to the culture of the lemon tree, but are well adapted

TABLE V

GENERAL CLASSIFICATION BY REGIONS OF SOUTHWESTERN U. S. WITH TYPICAL CITRUS VARIETIES AND DENSITY OF STOMATA

REGIONS	LOCALITIES	CLIMATICAL CONDITIONS	APPROXIMATE LATITUDE (N.)	TYPICAL CITRUS VARIETIES	STOMATA PER SQ. MM.
Desert regions (California and Arizona)	Imperial, Coachella, Salt River and Colorado River valleys	Intense summer heat; practically no rains	32°–33° 40′	Grapefruit (Marsh)	583
Great Interior Valley of California	Sacramento, San Joaquin	Hot and dry in summer, cold in winter; light to moderate rainfall	35°30′–40°30′	Navel orange	458
Southern Interior valleys of California	Riverside, Redlands, San Bernardino, Pomona	Moderately hot and dry in summer, some coastal influence in winter; medium rainfall	34°	Navel orange Valencia orange	458 504
Southern coastal region of California	San Diego, Orange, Los Angeles, and Santa Barbara counties	Cool in summer, rarely cold in winter; humidity usually high; light rainfall	32°30′–35°	Lemons (Eureka and Lisbon) Valencia orange	636–743 504

to the culture of grapefruit (central Florida), sweet oranges (Florida, southeastern China, Rhodesia, and Brazil), Satsuma (Japan), and shaddocks (Amoy). The cooler parts of the Temperate climate are those in which varieties of low stomatal density are cultivated, for example, Yuzu, Kansu, and Kunembo (Japan). The citrus districts of California and Japan which lie in the same latitude are marked by differences in their adaptability to varieties, which may

be due to the fact that one has a Mediterranean and the other a Temperate type of climatic factors.

The distribution of varieties of a single species also seems to be correlated with stomatal density. For example, four shaddocks (table III) are grown in the latitude between 20° N. and 21° N. The shaddock from Honolulu and the wild shaddock in the vicinity of the volcano Kilauea, near Honolulu, have higher density of stomata than those from Calcutta and Cuba, where there is much heavier precipitation. Three of the citron varieties, Indian citron, *C. medica* from the Philippine Islands, and Chinese lemon, are grown in the tropical zone. Precipitation during the spring months is higher in India than in China and the Philippine Islands, and the stomatal density appears to correspond to these differences in precipitation. The Italian citron, which has the highest density among the citrons, came from the typical Mediterranean climate.

In the *C. nobilis* (mandarin) group, Kunembo, Unshiu (Satsuma), Dancy, and King, which are low in stomatal density, thrive in the regions of spring and summer rains such as the northern coast of the Gulf of Mexico and southern Japan. It will be noted that the spring rainfall at Bartow, Florida, is greater than the total annual rainfall at Los Angeles. The orange varieties which have higher stomatal density, such as Ruby blood, St. Michael, Valencia, Jaffa, Navel, etc., seem to thrive in the localities where the climate is comparatively tropical (Rhodesia, Brazil, Canton, etc.), or in the dry so-called Mediterranean climate (coast of Mediterranean and southern California).

In connection with the relation of hardiness and stomatal density, it seems apparent that hardiness depends entirely upon the condition of the cell content, although there seems to be some coincidence of hardiness with density of stomata of the commercial species. Data given in table VI show some relation between stomatal density at Riverside, the latitude in which the variety or species is cultivated, and the hardiness of the tree with respect to low temperatures. With the exception of the lime (*C. aurantifolia*), the hardiness seems to be associated with low density of stomata. This relationship might be anticipated from the fact already mentioned, that species predominating in tropical regions of lower latitudes have a high

TABLE VI

RELATIVE DENSITY OF STOMATA IN RELATION TO HARDINESS TO COLD AND TO LATITUDE OF PRINCIPAL LOCALITY OF CULTIVATION

SPECIES	NO. OF VARIETIES STUDIED	AVERAGE STOMATA PER SQ. MM.	HARDINESS	LATITUDE (N.)	MAIN LOCALITY
C. webberi....	1	769		9–20°	Philippines
C. medica.....	4	673	Tenderest of all citrus	5–15°	Malay peninsula; Malabar coast
C. mitis.....	1	665		9–20°	Philippines
C. limonia.....	4	642	Somewhat tender	0–15° 37–38° 32–36°	Malay peninsula Sicily California
C. hystrix.....	2	627	Tender	0–23°	Tropical Pacific Island; Ceylon
C. maxima....	4	576	Moderately hardy	13–20° 24–27°	Bangkok, Siam Amoy, China
C. paradisi....	3	566	"	18–30°	Cultivated in West Indies and Florida
C. sinensis.....	6	484	"	23 16–23°* 26–30° 32–36° 37–44°	Canton Brazil Florida California Mediterranean region
C. nobilis.....	6	473	Moderately to very hardy; Satsuma is the hardiest of all edible oranges	King group: 28–30°	Chekiang, China; Florida
				Tangerine group: 25° 26–30°	 Fuchow, China Florida
				Satsuma group: 28–32° 31–35°	 Florida to Alabama Japan
C. aurantium..	5	454	Moderately hardy	26–30° 37°	Florida Seville, Spain

* S. latitude.

TABLE VI—*Continued*

Species	No. of varieties studied	Average stomata per sq. mm.	Hardiness	Latitude (N.)	Main locality
C. aurantifolia	4	453	Moderately tender; intermediate between citron and lemon	0–23° 9–20°	Malay to Canton Philippines
C. junos	2	416	Very hardy	31–40°	Japan
Fortunella margarita	1	461	"	31–40°	"
Poncirus trifoliata	1	495	"	31–40°	"

stomatal density, while those growing in the moist regions of higher latitudes have a low stomatal density.

Summary

1. Precipitation of rain during the spring months, while the young citrus leaves are developing, showed a stronger correlation with the number of stomata than the amount of annual rainfall; although it is impossible to disregard other factors, such as light, heat, and atmospheric humidity.

2. Species and varieties of the Aurantioideae show differences in the density of stomata which appear related to their place of origin. For example, most species growing in the tropics have more than 500 stomata per square millimeter, while those outside the tropics have a lower density.

3. Certain species, like *C. paradisi*, show adaptability to a wide range of conditions, growing both in the hottest districts and in the moist temperate region. Other species, like *C. limonia*, grow most successfully in regions having the Mediterranean type of climate.

4. The hardier varieties and species of citrus trees are characterized by low stomatal density, although there are a few well marked exceptions.

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SOME CARYOPHYLLACEOUS PLANTS INFLUENCED IN GROWTH AND STRUCTURE BY ARTIFICIAL ILLUMINATION SUPPLEMENTAL TO DAYLIGHT

FRANCIS RAMALEY

(WITH EIGHTEEN FIGURES)

Introduction

Since the appearance of the papers of GARNER and ALLARD (**9, 10**) dealing with photoperiodism, there has been a widespread interest in the relation of light to plant growth. The writer began experiments with maize and wheat in 1924, and in 1925 commenced a systematic study of the growth made by various plants when natural daylight is supplemented by artificial illumination. In earlier studies, the light was kept burning from late afternoon until 10:00 or 11:00 in the evening. After a time it became apparent that most plants do not need a rest period of darkness, and that more striking acceleration of growth takes place with longer illumination, although textbooks of plant physiology usually intimate or even definitely state that light has a retarding influence on growth. This may be true if light is very bright (**5**), but adding to the length of day with light of moderate intensity practically always stimulates stem elongation, and with most annuals this is accompanied by flower development. Beginning in 1926, the light was turned on each afternoon and kept burning until 8:00 the following morning. Since December, 1928, in all experiments the lights have been burned continuously, night and day. Progress reports of the study have been made at meetings of the Southwestern Division of the American Association for the Advancement of Science in Santa Fe (1927) and Flagstaff (1928), and a brief statement (**15**) has been published.

Earlier literature on the relation of light to plant growth has been well summarized by MACDOUGAL (**11**). More recent papers are noted by ADAMS (**1–4**), who found increased growth in height of most species when given supplementary artificial illumination, and by TINCKER (**17, 18**), who holds that "readiness to flower" is dependent

on the proper carbohydrate-nitrogen ratio, and that this is influenced by length of day, whether natural or artificial.

There is considerable difference in results of various workers who have experimented with lengthened daily illumination, even when methods employed were essentially the same. Probably apparent contradictions result from the use of plants with widely different inherent qualities, some responding readily to the longer light period, and others being so established in their reactions that no profound changes can be brought about. With the thought of using plants of somewhat like nature, the present study has been confined to a single family, although the writer has also undertaken work with many other genera and families.

Material and methods

The uniform procedure has been to plant seeds in 5-inch pots (at least four pots for each species), and when the seedlings were well established to place a part of the material under electric lights, and to put the rest a few feet away on the same greenhouse bench but shielded from the direct rays of the lamps. All plants received full natural illumination, while those of the experimental series had, in addition, light for 24 hours per day from two 100-Watt Mazda lamps suspended in a reflector above them. The lamps do not produce any measurable increase of temperature. Use of a foot-candle meter at night showed the light to be from 10 to 20 foot-candles on the various experimental plants, a few of the tallest receiving twice this illumination at their growing tips. The controls, being in the same room, were subject to faint diffuse light at night, but measurement showed that this was less than one-half candle. There was no indication that such very weak light had any influence on the growth of the check plants. The soil used was a sandy loam; all plants were carefully tended. A temperature of 60°–80° F. was usually maintained. The less vigorous seedlings were pulled up and from four to eight plants left in each pot, except with larger species when only two or three were permitted to grow. In recording measurements the averages have been used; for anatomical study the most advanced individuals have been taken, and these have been compared with the most advanced of the control series.

Growth, flowering, and external appearance

Flowering occurred earlier in the plants given supplementary illumination. Often the stems of the experimental plants were slender and weak, sometimes decumbent; in all cases roots showed poor development. The plants were at every stage taller than the controls. Table I summarizes observations on ten species. It is seen that artificial illumination forces quickly into bloom during the short

TABLE I

RECORD OF PLANTS GROWN WITH SUPPLEMENTARY ARTIFICIAL LIGHT

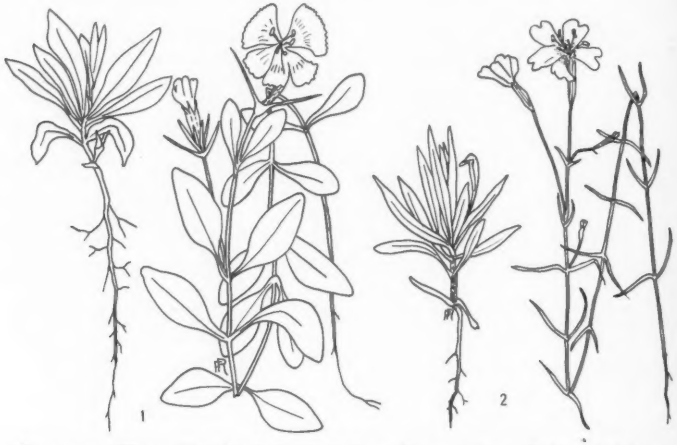
SPECIES	DATE PLANTED	EXPERIMENT STARTED	PLANTS BLOOMED	CONTROLS BLOOMED	ACCELERATION IN DAYS	REMARKS
Agrostemma coeli	11/8/30	11/25/30	1/10/31	4/18/31	98	Controls did not bloom until lengthening days of spring; experimental plants decumbent
Dianthus barbatus	12/28/30	1/18/31	3/18/31	5/2/31	45	Experimental plants weak; leaves pale
D. caryophyllus	9/16/28	9/30/28	2/10/29	3/3/29	21	All plants sturdy; much alike
D. chinensis heddewigii	12/25/27	1/4/28	3/18/28	4/21/28	34	Experimental plants procumbent
D. plumarius	11/27/30	2/17/31	4/5/31	5/1/31	26	All plants much alike
Gypsophila paniculata	12/25/27	1/4/28	2/12/28	3/18/28	36	Experimental plants look like different species; leaves small and thin
Saponaria multiflora	1/18/31	2/2/31	3/12/31	4/28/31	46	All plants much alike
Silene acaulis	4/5/28	11/22/28	.....	.....	.....	Stems and leaves of experimental plants long and slender; none of plants bloomed
S. inflata	3/18/29	3/31/29	7/18/29	.....	.....	Leaves of experimental plants small; controls did not bloom the first season
Viscaria viscosa	11/15/30	11/27/30	1/10/31	5/10/31	120	Leaves of experimental plants very small; controls did not bloom until lengthening days of spring

days of winter *Agrostemma coeli*, *Dianthus barbatus*, *Saponaria multiflora*, and *Viscaria viscosa*. Although not used in these experiments, the common weed *Alsine media* was observed to be unaltered by the artificial light, although reported (**13**) to develop well marked hairiness when grown close to a 200 candle-power lamp. Not one of the species used showed any modification in pubescence or anthocyan development, but the vegetative parts often exhibited a degree of paleness. Flowers were exactly the same as under ordinary greenhouse conditions.

Anatomy

Agrostemma coeli (figs. 3–6) was studied when 96 days old, the experimental plants being under electric light for 79 days and until

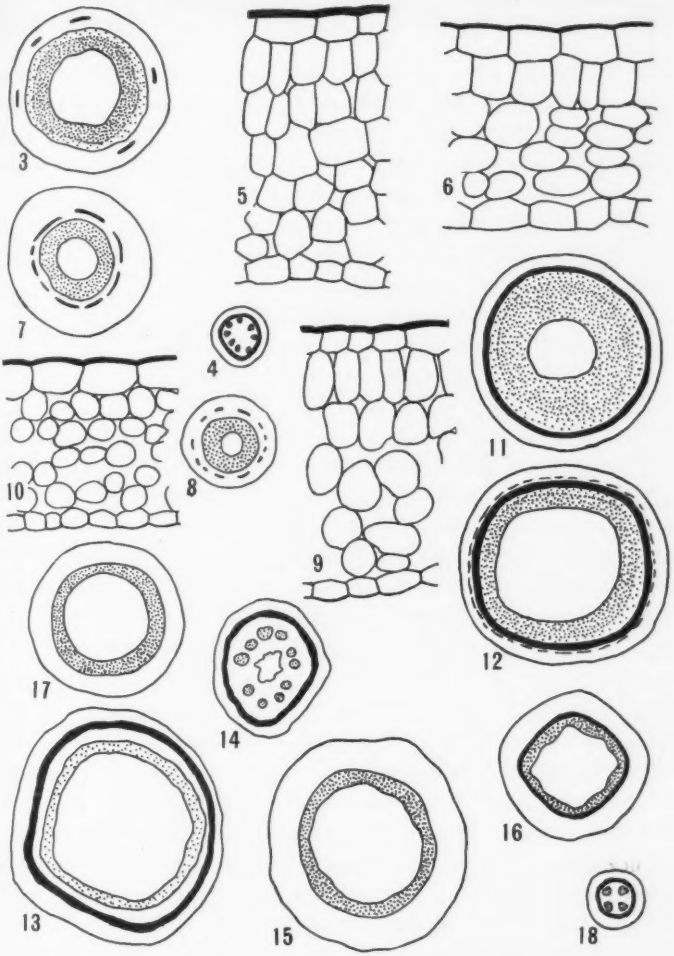
in full bloom. In cross-section of the third internode of the stem the experimental plants have a diameter 41 per cent that of the controls; the pericycle forms a continuous cylinder of lignified sclerenchyma instead of having, as the controls, scattered groups of sclerenchyma cells; vascular tissue is found in about twelve separate bundles; starch is absent from pith and cortex. Leaves of experimental plants are thin, about 66 per cent of thickness of controls.



FIGS. 1, 2.—Fig. 1, *Dianthus barbatus*, 80 days old: experimental plant at right, control at left. Fig. 2, *Viscaria viscosa*, 56 days old: experimental plant at right, control at left; ×1:3.

Dianthus barbatus (figs. 1, 7–10) was studied when 80 days old, the experimental plants being under electric light for 70 days and until in full bloom. In cross-sections of the third internode of the stem these plants have a diameter 51 per cent that of the controls; both sets of plants have interrupted sclerenchyma in the pericycle with beginning of cork formation; leaves of experimental plants are thin, being 78 per cent as thick as the controls and with no true palisade.

Dianthus plumarius (figs. 11, 12) was studied when 93 days old, the experimental plants being under electric light for 42 days and until in full bloom. In cross-sections of the third internode the stems of these plants and of controls are of the same diameter, but the



FIGS. 3–18.—Figs. 3–6, *Agrostemma coeli*, 96 days old: 3, cross-section of stem of control plant; 4, corresponding internode of experimental plant; 5, vertical section of leaf of control; 6, leaf of experimental plant. Figs. 7–10, *Dianthus barbatus*, 80 days old: 7, cross-section of stem of control plant; 8, corresponding internode of experimental plant; 9, vertical section of leaf of control; 10, leaf of experimental plant. Figs. 11, 12, *Dianthus plumarius*, 93 days old: 11, cross-section of stem of control; 12, same of corresponding internode of experimental plant (circumferential row of dashes indicates beginning of cork formation). Figs. 13, 14, *Gypsophila paniculata*, 111 days old: 13, cross-section of stem of control plant; 14, same of corresponding internode of experimental plant. Figs. 15, 16, *Saponaria multiflora*, 53 days old: 15, cross-section of stem of control; 16, same of corresponding internode of experimental plant. Figs. 17, 18, *Viscaria viscosa*, 56 days old: 17, cross-section of stem of control; 18, same of corresponding internode of experimental plant. Vascular tissue shown by dotting; stereome is black. Magnification of stem cross-sections, about ×16; leaf sections, about ×160.

controls have twice as much vascular tissue and a somewhat thinner sclerenchyma ring of pericycle; xylem of experimental plants has larger and more numerous vessels. There is the beginning of cork formation in the lower layers of the cortex in the experimental plants.

Gypsophila paniculata (figs. 13, 14) was studied when 111 days old, the experimental plants being under electric light for 90 days and until in full bloom and fruit. In cross-sections of the third internode of the stem the experimental plants have a diameter 55 per cent that of the controls; both stems have a continuous ring of stereome, the controls possessing undissected xylem and phloem cylinders while the vascular tissue of the experimental plants is in distinct bundles and the pith is hollow.

Saponaria multiflora (figs. 15, 16) was studied when 53 days old, the experimental plants being under electric light for 38 days and until in full bloom. In cross-sections of the second internode the stems have a diameter 68 per cent that of the controls; when studied later (at 94 days) the controls had so increased in diameter that the experimental plants were only 51 per cent; a pericyclic stereome ring is present early, while this does not develop until later in the control plants. No starch was recognized in the experimental plants although abundant in the controls. Leaf structures show no differences.

Viscaria viscosa (figs. 2, 17, 18) was studied when 56 days old, the experimental plants being under electric light for 44 days and until in full bloom. In the third internode the stem diameter is only 31 per cent that of the controls; there are four small vascular bundles and a thick pericyclic stereome cylinder, whereas the controls have undissected vascular tissue and no stereome at all in the present stage. When examined a month later (at 86 days) the stems of the controls were thicker, with a strong development of xylem and the beginning of cork formation in the cortex; the experimental plants had increased only slightly in thickness and there was no change in the vascular tissue or stereome. Starch was abundant in the cortex and pith parenchyma of the controls.

Discussion

In studies to determine the influence of supplementary long-day lighting, some experimenters (5) have employed a powerful illumi-

nation nearly equal to that of sunlight; others (**1, 4, 17, 18**) have used such light as would be satisfactory for reading. The physiological problems involved are not exactly the same in the two kinds of experiments, yet results are often much alike. For forcing plants to develop and flower on short notice, whether for laboratory use or for sale, a knowledge of behavior with moderate light intensities is especially valuable since the necessary equipment is inexpensive and easily provided. It is shown in the present study (table I) that certain species respond quickly and very satisfactorily to illumination of the lesser intensities.

It was long ago noted by BONNIER (**6**) that plants given electric illumination in addition to daylight showed a somewhat etiolated appearance, having long internodes and small leaves. In the present study this condition is plainly evident in *Agrostemma*, *Gypsophila*, and *Viscaria*, but not in *Dianthus plumarius*, *D. caryophyllus*, or *Saponaria multiflora*. The other Caryophyllaceae of the experimental series exhibit an elongation of stems to some degree but their leaves are normal. Electrically lighted plants of *Gypsophila* and *Viscaria* might easily be taken for species different from their controls.

It is often held that in experiments with different day lengths, the growth of roots parallels that of shoots; in the present study the long-day exposure results in plants with lengthened stems but with short and slightly branched roots. One experimenter (**7**) finds that long daylight exposures favor development of roots in the radish. Many workers recognize that an accumulation of carbohydrates is accompanied by tuberization or by root thickening, hence it is perhaps to be expected that in these experimental plants, which are nearly all starch-free, the underground parts will be poorly developed. A small amount of vascular tissue, especially the phloem, is a striking feature. The slight phloem development must of necessity result in poor root nutrition and rather inadequate root growth.

Stems of the plants which have been given the extra illumination are generally weak, presumably because of their small diameter, although strengthening tissue frequently is more prominent than in

the controls. The long-night exposure to weak light no doubt causes thinness of stem, as noted in experiments by others (**11, 14**).

Leaves of plants belonging to the experimental series are sometimes but not always thinner than leaves of control plants, yet it has been reported (**8**) that, in tomatoes and peppers, leaves of plants given additional light were thicker than those of controls. In the present study the leaves of experimental plants tend to approach the usual shade-leaf type of structure, with a single layer of palisade, general loose arrangement of cells, and thin spongy layer; but instead of being a dark intense green they are usually somewhat pale. These plants resemble in general those which PFEIFFER (**12**) grew in light from which the violet rays were eliminated.

Explanation of the peculiarities of plant growth in the present experiment involves many factors. The plants received little violet and ultra-violet radiation because grown under glass, but the controls likewise were deprived of the short waves of the spectrum which seem to retard growth. The Mazda lamps furnished a large proportion of light of the longer wave lengths, and these rays may perhaps be responsible for some of the elongation of internodes, for it has been shown (**16**) that red rays favor the lengthening of stems while yellow and green rays bring about greater chlorophyll formation; it will be remembered that the plants here treated were often pale in color. Apparently the modifications in plant structure are due partly to the weak night-illumination and partly to the quality of the artificial light which was furnished.

The energy of the electric light (10–20 foot-candles) would seem almost negligible in comparison with daylight of 2000–4000 foot candles, yet this amount of light was sufficient to bring the plants out of the rosette stage and to cause early flowering. During the night hours of weak illumination, the experimental plants grew in length and used up carbohydrate food without carrying on photosynthesis to any extent. They thus became starved and could not develop the stem diameter, nor, in some species, the leaf size and thickness of the controls.

Summary

1. Eight species of Caryophyllaceous plants were grown from seed to maturity in the greenhouse, the natural daylight being supple-

mented by continuous electric light from incandescent bulbs furnishing 10–20 foot-candles.

2. The experimental plants grew taller than the controls, blossomed earlier, often had more slender stems with the vascular tissue, especially the phloem, weakly developed. Roots tended to be small and short; starch was generally absent from pith and cortex of the stem. Leaves were sometimes but not always thinner than those of the controls, and sometimes showed a single layer of palisade as in rather typical shade plants.

3. Certain species of *Agrostemma*, *Dianthus*, and *Viscaria* were brought into bloom very quickly, even during short winter days, by the use of continuous supplementary light.

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INCREASE OF SUGAR UTILIZATION IN SPIROGYRA BY MEANS OF COMMERCIAL FERTILIZERS

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D. J. VERDA, AND W. E. BURGE

LAVOISIER (1) was the first to show that food materials are burned in the body with resulting heat production. He also found that ingestion of food increased oxidation. RUBNER (3) showed that ingestion of meat increased heat production more than did ingestion of fats or carbohydrates. The stimulating effect of meat or protein on metabolism is due to the amino acids, the end products of protein digestion as shown by LUSK (2). SPOEHR and MCGEE (4) found that the amino acid, glycine, increased sugar utilization in *Helianthus*.

The object of the present investigation was to determine the effect of fertilizers on the rate of sugar utilization in the plant. *Spirogyra porticalis* was selected for the experiment. Large quantities of the material were collected from a nearby lake and brought to the laboratory. It was then washed in 0.1 per cent dextrose solution, and after removing the excess liquid by gently squeezing with the hands, lots of 150 gm. each were weighed out. These batches were placed in flat bottomed dishes, 20 cm. in diameter, in 600 cc. of 0.1 per cent dextrose solution. Six hundred mg. of 53 different commercial fertilizers were weighed out and boiled in 15 cc. of water.[1] After cooling, these fertilizers were added to the different batches of *Spirogyra* immersed in the sugar solutions. Portions of material to which no fertilizers were added served for controls. A small amount of sugar solution was removed from each of the dishes containing the *Spirogyra*, and sugar determinations were made according to the method of BENEDICT, immediately and after 40 hours, at the end of the experiments. Before making the determinations at the end of the experiments, water was added to make up for that lost by evaporation.

The results of an average of five series of experiments are shown in table I. By comparing the percentages of sugar used, it will be

[1] We desire to express thanks to the different fertilizer companies who so generously supplied us with the samples of fertilizers used in this investigation.

TABLE I

RESULTS FROM ADDITION OF DIFFERENT COMMERCIAL FERTILIZERS TO SPIROGYRA IN SUGAR SOLUTION

	PERCENTAGE OF SUGAR USED								
0	10	20	30	40	50	60	70	80	90
Control			32						
+Leunasalpeter									90
+Arcadian Supreme Nitrate of Soda									89
+Urea Calcium Nitrate							79		
+Plant Marvel "B"							73		
+Calurea 34.5% Nitrogen							69		
+Multiple Superphosphate 40%							68		
+2–8–10 Synthetic Nitrogen							68		
+Swift's Red Steer 20% Superphosphate							67		
+20% Superphosphate							67		
+Wizard Shredded Cow Manure							65		
+2–8–10							63		
+0–5–10							63		
+0–12–12							62		
+Swift's Red Steer 0–8–24							62		
+Wizard Pulverized Mule Manure							62		
+0–5–15							62		
+Bone-Meal							62		
+Ammonium Sulphate							62		
+Wizard Pulverized Sheep Manure							61		
+Sheep's Head Pulverized Sheep Manure							61		
+4–24–4 Multiple Strength							61		
+0–20–20							61		
+3–8–6							61		
+V. C. 2–12–2							61		
+Sulphate of Potash							60		
+4–16–20							60		
+0–10–10							60		
+Ammonium Sulphate							60		
+Plant Marvel "C"							60		
+V. C. 4–16–4							60		
+2–8–16							60		
+2–12–6							60		
+2–8–16							60		
+V. C. Superphosphate 24%						59			
+V. C. Superphosphate 16%						58			
+0–12–12						58			
+0–8–24						58			
+Swift's Red Steer 4–16–4						58			
+Urea Basf 46% Synthetic N						57			
+V. C. 2–10–6						57			
+V. C. 3–12–4						57			
+V. C. 4–24–4						57			
+Swift's Red Steer 2–16–2						57			
+Armour's Big Crop 24% Superphosphate						56			
+Sure Winner 20% Phosphate						56			
+29% Superphosphate						54			
+16–9–0						54			
+20% Superphosphate						54			
+0–9–27						53			
+Muriate of Potash						52			
+2–12–6						52			
+Wizard Pulverized Hog Manure					45				
+1–10–4					43				
Control			32						

seen that all the fertilizers increased sugar utilization above the controls; that fertilizers such as Leunasalpeter and Arcadian Supreme Nitrate of Soda increased it most, while Muriate of Potash, 2–12–6, Wizard Pulverized Hog Manure, and 1–10–4 increased it least.

The next experiments were carried out with the use of chemical compounds, many of which are contained in the fertilizers used. The method of procedure was the same as that already described.

TABLE II

RESULTS FROM ADDITION OF CHEMICALS TO SPIROGYRA IN SUGAR SOLUTION

	PERCENTAGE OF SUGAR USED								
0	10	20	30	40	50	60	70	80	90
Control				41					
+CO $(NH_2)_2$									85
+$(NH_4)_2SO_4$							66		
+$Ca_3(PO_4)_2$							64		
+K_2HPO_4					56				
+NaCl					53				
+KCl					53				
+$(NH_4)_2HPO_4$					51				
+$CaSO_4$					51				
+K_2SO_4					50				
+$KHCO_3$					49				
+$Ca(NO_3)_2$					47				
+$NaNO_3$					46				
+$MgSO_4$					42				
+$MgCl_2$					42				
+Na_2HPO_4					41				
+$CaCl_2$					39				
+Na_2SO_4					38				
+$CaH_4(PO_4)_2$					36				
+$NaHCO_3$					29				
Control					41				

Lots of *Spirogyra* weighing 150 gm. were placed in 600 cc. of 0.1 per cent dextrose solution in dishes similar to those previously used. To the different dishes, 600 mg. of the various chemicals listed in table II were added, after being dissolved in 10 cc. of boiling water. After adding the chemicals, small amounts of the sugar solution were removed from the dishes and sugar determinations were run immediately and after 48 hours, at the end of the experiments. The results of an average of four series of experiments are shown in table II. By comparing the percentages of sugar used by the various lots of *Spirogyra*, it will be seen that those to which such chemicals as urea, ammonium sulphate, and calcium phosphate were added used most

sugar, while those to which such chemicals as calcium chloride, sodium sulphate, and sodium bicarbonate were added, used least.

It may be noticed in tables I and II that the fertilizers and pure chemicals that stimulated sugar utilization most were those richest in nitrogen. Table III gives data taken from table II, showing a comparison of nitrogen content and stimulating effect on sugar utilization. It will be seen that urea, the richest compound in nitrogen (46 per cent), increased sugar utilization most, while calcium and sodium nitrates, the poorest compounds in nitrogen (13 and 16 per

TABLE III

RELATION BETWEEN NITROGEN CONTENT AND STIMULATING EFFECT ON SUGAR UTILIZATION

	PERCENTAGE OF SUGAR USED								
	0	10	20	30	40	50	60	70	80	90
Control	41									
$+CO(NH_2)_2$ (46% N)	85									
$+(NH_4)_2SO_4$ (21% N)	66									
$+(NH_4)_2HPO_4$ (21% N)	51									
$+NaNO_3$ (16% N)	46									
$+Ca(NO_3)_2$ (13% N)	47									

cent, respectively), increased it least; and that ammonium sulphate and phosphate, which contain intermediate amounts of nitrogen, had intermediate effects on the rate of sugar utilization.

It should be stated that the experiments reported in this paper were carried out in dim light. After certain of the experiments had been going for several hours, small portions of sugar solution were removed from the dishes and sugar determinations made. The *Spirogyra* was then removed from the dishes, and it was found that sugar utilization was greatly decreased or had practically ceased upon this removal, indicating that bacteria were not using the sugar. In what way the *Spirogyra* utilizes the sugar, whether for storage, metabolized or adsorbed, the experiments do not indicate. Apparently they do show, however, that the *Spirogyra* and not the bacteria used the sugar, and that fertilizers increased the rate of utilization.

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ORIGIN OF LEAF, AND ADVENTITIOUS AND SECONDARY ROOTS OF CERATOPTERIS THALICTROIDES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 423

M. DORISSE HOWE

(WITH NINE FIGURES)

According to the classification of ENGLER, *Ceratopteris thalictroides* is a member of the Parkeriaceae. This tropical amphibious form has the peculiarity of forming buds in the notches of the leaves, which rapidly develop into new plants while the leaves still form a part of the parent plant. It was these buds which furnished material for the following work. The plants were growing in the University of Chicago greenhouse. Material collected during a time of rapid growth was found most favorable for the study of origins. Formalin-acetic-alcohol proved to be a satisfactory killing and fixing agent. Sections were stained with safranin and light green.

The general anatomy of *Ceratopteris thalictroides* has been studied by Miss FORD[1]. Her conclusions, however, were not of a nature to be of interest in this paper. The origin and development of the leaf were briefly described by KNY[2]. He mentioned that the root is exogenous, but beyond stating that it is derived from a hypodermal cell he did not describe its origin.

The leaf initial is derived from the outside portion of the segment of the tetrahedral apical cell of the main axis. The first division of the segment forms a radial anticlinal wall by which the segment is divided into two unequal cells (fig. 1 *a*). The first division of the larger of these two cells is periclinal, giving rise to a large outer and a small inner cell (fig. 1 *b*). The large outer cell then undergoes an anticlinal division in a plane parallel to the upper wall of the segment. The wall formed at this division curves downward at the

[1] FORD, S. O., Anatomy of *Ceratopteris thalictroides*. Ann. Botany **26**:95. 1902.

[2] KNY, L., Die Entwickelung der Parkeriaceae. Nova Acta K. Leop. Deutsch. Akad. Naturf. 1875.

inner edge (fig. 1 *c*). The upper cell thus formed is narrow, the lower one broad. This latter undergoes a division similar to the preceding one, except that the wall formed curves upward at the inner edge (fig. 1 *d*). By the last two divisions a wedge-shaped cell is formed, which is the leaf initial. The leaf initial divides first by a radial anticlinal wall which curves to the right or left as seen in cross-section (fig. 1 *e*). If this wall comes in first on the right, the wall curves to the left at its inner edge. When seen in face view the same wall bends to the right. The next division of the leaf initial is similar to the one preceding it (fig. 1 *f*), so that as a result of these two divi-

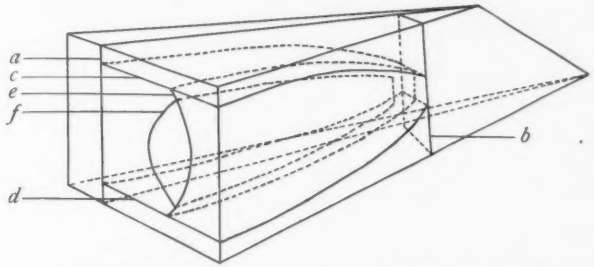
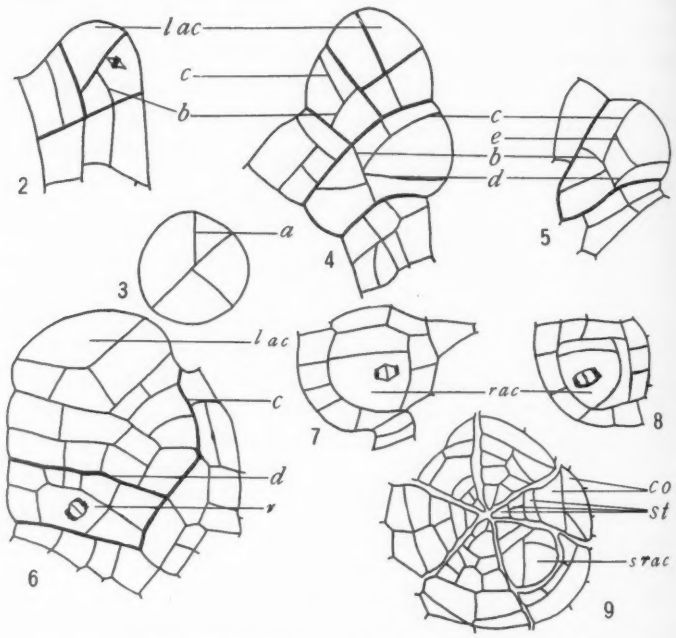


FIG. 1.—Diagram in three dimensions of segment of apical cell of main axis

sions a dolabrate cell is formed which functions as the apical cell of the leaf.

The root initial of the first adventitious root at the node is derived from the cell immediately below the leaf initial. This cell undergoes a periclinal division by which an epidermal cell is cut off on the outside. The root initial is derived from the inner cell thus formed. By one anticlinal division parallel to the upper wall of the original segment, and one periclinal division in addition to one or more radial anticlinal divisions, a mass of cells is formed from this inner cell. The root initial is the lower of the row of two cells next to the epidermis (fig. 6 *r*, *i*). Thus the root is not derived from the leaf itself, but from the same segment as is the leaf. The root initial may be irregular or roughly cubical. By its first three divisions, which take place in a clockwise direction on the inner faces of the cell, the root initial is transformed into a tetrahedral cell with one face toward the outside of the stem (fig. 7). The next three divisions take place

in the same planes as do the first three, and in the same order. The following division of the cell is periclinal, cutting off the root cap (fig. 8).



FIGS. 2–9.*—Fig. 2, longitudinal section of tip of main axis showing segment divisions; fig. 3, transverse section of tip of main axis; fig. 4, longitudinal section of main axis showing leaf initial; fig. 5, portion of longitudinal section of main axis showing cells derived from one segment; fig. 6, longitudinal section of leaf and portion of main axis showing root initial; fig. 7, portion of longitudinal section of main axis showing second division of root initial; fig. 8, portion of longitudinal section of main axis showing apical cell of root cutting off first cap cell; fig. 9, portion of transverse section of young root showing origin of secondary root; all ×275.

* *a*, wall formed by first division of segment; *b*, second wall; *c*, third wall; *d*, fourth wall; *e*, fifth wall; *f*, sixth wall; *sac*, stem apical cell; *lac*, leaf apical cell; *r*, root initial; *rac*, root apical cell; *co*, cortical cells; *st* cells of stelar region; *srac*, secondary root apical cell.

The secondary root initial appears as an enlarged cell of the inner layer of the cortex of the primary root, near the apical cell. This layer finally occupies a position which is usually occupied by the

endodermis. Subsequent behavior of the secondary root initial is the same as that of the primary root initial.

The first root at a node arises from the products of the cell immediately below the leaf initial. Other roots arise soon after the first one, some of which appear to come from cells derived from the leaf initial itself.

Summary

1. In *Ceratopteris thalictroides* the leaf initial develops from the outside portion of the segment of the apical cell of the main axis.

2. The initial of the first adventitious root at the node develops from a hypodermal cell derived from the cell immediately below the leaf initial. Later roots may develop from cells derived from the leaf initial. These roots are also hypodermal in origin.

3. The secondary root develops from a layer occupying the position of the endodermis in the primary root.

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CURRENT LITERATURE

BOOK REVIEWS

Plant physiology

Botanists, and especially plant physiologists, will welcome the publication of MILLER's book on plant physiology.[1] The volume is intended for upper classmen and graduate students as well as for research workers in plant physiology. The whole field of the physiology of the green plant is covered. The order of treatment of the various subjects is logical. At the beginning are two chapters of a general nature, in which is given the description of the parts of the plant cell and the relations of the cell membranes to permeability and the development of osmotic pressure. Then follow chapters on roots; intake of water and solutes; the essential elements; transpiration; synthesis of carbohydrates; nitrogen and fat metabolism; digestion, including enzyme action; translocation of water, inorganic compounds, and organic compounds; respiration; and growth.

The book serves as a source of information, not only concerning American investigations, but of the investigations of English and continental workers. There has long been a need for a book of this kind. The European texts discuss the continental investigations but do not summarize adequately English and American research work. The material is discussed in an unbiased manner, the various theories and shades of opinion being given. Citations of the work summarized are given at the end of each chapter, the citations being referred to by years in the text. Thus the student will be enabled to read further on any subject in which he is especially interested. One is impressed with the great number of references discussed or referred to in the text. The citations at the end of each chapter cover several pages.

In the case of a book dealing with a subject which is changing as rapidly as is plant physiology, and concerning various phases of which investigators hold such divergent viewpoints, it is too much to expect that the author's treatment will be approved in all respects by plant physiologists. In regard to certain subjects, it seems to the reviewer to be inadequate or misleading. For example, it appears unnecessary to divide absorption of water by roots into absorption due to imbibition and osmotic pressure and "passive absorption," by the latter meaning that the saturation deficit set up in the leaves by transpiration is transmitted back through leaf, stem, and root to the soil, thus causing the entrance of water. It seems better to regard imbibition and osmotic pressure as involved in

[1] MILLER, E. C., Plant physiology with reference to the green plant. pp. xxiv+900. *figs. 38.* McGraw-Hill Book Co., New York. 1931.

the entrance of water into the root and its passage through the cortical cells of the root, both when transpiration is active and when conditions are such as to prevent transpiration or to retard it considerably. In discussing the functions of transpiration, the fact that increasing transpiration does not cause greater absorption of solutes by the root is given as evidence against the importance of transpiration in the transference of salts from the root to the leaves; but since solutes and water are absorbed by the root independently, and moved independently through the cortical cells to the root tracheae, and since a slow rate of transpiration is probably about as effective as a high one in keeping the concentration of salts in the tracheal sap low, this argument would seem to be invalid. The transpiration stream cannot carry away any more solute material than reaches it by diffusion through the root cortex. One of the most interesting phases of fat and protein metabolism is seen in their energy relations. This is rather inadequately treated in the book.

The volume is well provided with features enabling the reader readily to find information on any subject in which he is interested. There is both a subject and an author index. In addition, the table of contents is exceptionally full, each subject being divided in a detailed manner. The book is one which no plant physiologist can afford to be without.—S. V. EATON.

Principles of plant biochemistry

The first volume of a two-volume work on the principles of plant biochemistry has been published by Mrs. ONSLOW,[2] well known among plant physiologists and biochemists for her work on the anthocyanin pigments of plants, her practical plant biochemistry, and for her research on the oxidase system of plants. The new work gives an introduction to the carbohydrates, proteins, and the oxidizing systems of plants. The two chapters on carbohydrates are on the sugars and the cell wall. There is a chapter on oxidizing and reducing systems of plants, and then two chapters on the nitrogenous constituents. The first is entitled the plant proteins, and the second, nitrogen metabolism. The final chapter is on respiration.

The chapter on sugar considers those types found in the higher green plants, mono-, di-, tri-, and tetra-saccharides, and the interrelations of these sugars in metabolism. There are useful diagrams to assist the student in grasping these relationships. The latter part of the chapter discusses the first sugar of photosynthesis (problem not solved), the glucose-fructose ratio in the plant, the concentration of hexoses, and the formation of starch and sucrose.

The polysaccharides and their congeners are considered in the chapter on the cell wall. This includes: celluloses, such as true cellulose, hydro- and oxycellulose, hemicellulose, pectic substances, gums and mucilages, cutin, cutocellulose, suberin, and lignins. One wishes that more were known of the actual

[2] ONSLOW, MURIEL WHELDALE, Principles of plant biochemistry. 8vo. pp. 326. University of Cambridge Press. 1931.

"ontogeny" of these substances; that is, the actual metabolic reactions by which they arise.

The chapter on oxidizing and reducing systems is devoted mainly to the oxygenase-peroxidase systems, tyrosinase, laccase, oxido-reductases, etc., and their relation to respiration. The reviewer thinks it would have been a better arrangement of chapters to have placed this just before the final chapter on respiration, instead of between the work on carbohydrates and nitrogenous compounds.

The chapter on plant proteins includes the albumins, globulins, prolamins, and glutelins, with tables listing those that have been isolated. There is also a table of hydrolytic products of the proteins. Under nitrogen metabolism the author presents a general summary, and specific accounts of metabolism in seedlings, ripening seeds, in starved leaves and shoots, etc. Protein synthesis is discussed, also the decomposition of amino acids by oxidation, synthesis of amino acids, and a survey of recent studies of nitrogen metabolism. The chapter closes with a consideration of purine metabolism, proteolysis, and the amino acids thus produced.

The final chapter deals with the metabolism and fermentation by yeast, and the respiration of higher plants. Here the author considers the respiratory substrate, the relation of phosphate to respiration, the dismutation of methylglyoxal, acetaldehyde as an intermediate product, and the coenzymes of the various steps. The fate of acetaldehyde, the part played by molecular oxygen in the process, and oxidative anabolism are discussed in the final sections.

While the work seems choppy in places, and not thoroughly digested, it is a valuably summary, particularly of the English and continental European work. Eleven bibliographies are provided for wide reading in that field. The second volume will be awaited with interest.—C. A. SHULL.

Handbook of plant analysis

Another excellent handbook for the research plant chemist and physiologist has just appeared. Under the leadership of KLEIN,[3] the methods of examining plant materials have been brought together by a group of about a dozen collaborators. The first volume of the set is devoted to the general methods of examination for quantitative information along many lines, rather than to the methods of organic analysis for the materials of metabolism. The latter phase of analysis will no doubt be covered in the succeeding volume.

The introductory section deals with the problems of testing reagents for purity. These reagents are listed alphabetically for ease of reference, and the impurities frequently found in them are mentioned, with methods of detection and removal. Following this section, the general methods of examination of plant materials are presented in seventeen sections, the first of which takes up such

[3] KLEIN, G., Handbuch der Pflanzenanalyse. Vol. I. 8vo. pp. xii+627. Julius Springer. Vienna. 1931.

problems as weighing, incinerating, heating, cooling, evaporating, concentrating of extracts, drying, recrystallizing, washing, stirring and shaking, clearing and decolorizing, etc. The next section gives methods of filtration, centrifuging, ultrafiltration, dialysis, electrodialysis, extraction by use of solvents, distillation, and sublimation. The succeeding sections cover the methods of qualitative elemental analysis; quantitative micro-analysis for the elements; determination of general group and radical characteristics ($-OH$, $-COOH$, $=CO$, $-NH_2$, etc.); molecular weight determinations and deduction of chemical formulae; gravimetric and volumetric methods; general physical methods (specific gravity, melting and boiling points, solubility, viscosity, molecular weights); optical methods (such as polarization, refractometry, interferometry, spectroscopy, spectrophotometry, colorimetry, and nephelometry); fluorometry; fluorescence; ultramicroscopy; photochemical analysis; electrical conductivity; electrometric determination of hydrogen-ion concentrations; colorimetric hydrogen-ion measurements; and calorimetry.

The foregoing eighteen sections comprise the first division of the subject, on general chemical and physical methods. The last portion of the first volume falls in the second division of the handbook, which deals with the general manipulations and complete analysis of plants. Only two sections of this division are included in volume I. The first of these takes up such manipulations as sampling, preservation, stabilization and drying of materials, grinding, expression of juice, steam distillation, and preparation of plant extracts by such processes as maceration, digestion, percolation, extraction, and shaking out. Solvents are considered in these connections. The second and final section deals with preliminary examination as to dry weight and water content, ethereal oil content, ash, and extractives; and the beginnings of the more thorough analysis, as microsublimation, preparation and testing of petrolether extracts, water extracts, and the examination of steam distillates, extracts, press juice, and inorganic constituents.

The work covers a very wide range of research methods, and will be useful to a wide circle of investigators in medicine, biochemistry, organic chemistry, pharmacology, agricultural chemistry, food chemistry, and plant physiology. Anyone who must make careful examination of plant materials of any kind will want this handbook as a guide. There are 323 figures in the text, and a rather comprehensive index. It is a welcome addition to the source books on methods of research in the botanical sciences.—C. A. SHULL.

Fruit culture

A new textbook of fruit culture has been prepared by KOBEL,[4] which stresses the physiological side of horticultural science. The first section of the work deals with the general physiology of fruit trees, such as the absorption of water from the soil, transpiration and water transport, intake and utilization of min-

[4] KOBEL, F., Lehrbuch des Obstbaus auf physiologischer Grundlage. 8vo. pp. viii+274. *Figs.* 63. J. Springer. Berlin. 1931.

eral nutrients, photosynthesis, deposition and use of food reserves, effects of cold and heat, and vegetative growth. The author then considers the problems of fruit bud formation and their development up to winter condition, theories of causes of flower bud formation, and methods of influencing this bud development by cultural practices, such as fertilizing the soil, combining stocks and scions of differing vigor, accelerating or retarding synthesis of carbohydrates, girdling and strangling of branches, and pruning. Periodicity of bearing is briefly treated.

The third section considers the opening of flowers, fruit setting from fertilization, fruit setting without fertilization, and the development of the fruit from the beginning to the ripe stage. Under fruit setting from fertilization, the author describes the normal processes of pollen development, female gametophyte, fertilization, and embryo formation to the seed stage. Departures from the normal behavior are considered, with special reference to sterility, including sterility due to morphological conditions, pollen sterility, sterility of female sex cells, formation of sterile seeds, and self- and inter-sterility of the various specific fruits, apples, pears, quinces, sweet and sour cherries, plums and prunes, apricots, peaches and almonds.

Fruit development includes the problem of the "June drop," and the physiological changes of the normal fruit during growth and ripening. The influence of external factors on ripening processes is discussed, and the influence of seed number upon fruit size and quality.

The fourth section is an interesting discussion of the relations between vegetative growth, flower bud differentiation, and fruiting. There are only about 20 pages in this section, but it is a valuable summary of this important phase of fruit physiology. The final section is devoted to the problems of improvement of fruits by breeding and selection.

The presentation is relatively clear and simple, and students will find it a valuable guide to an appreciation of the physiology of the fruit-bearing section of our economic plants, particularly the tree fruits.—C. A. SHULL.

Environment and plant development

The field of physiological ecology has been developing rapidly during the past decade, and among the European investigators in the experimental phases of the subject no one has stood higher than LUNDEGÅRDH. Perhaps his most notable contributions have been in establishing the relations existing between the rate of photosynthesis and the different degrees of light intensities. His curves showing the different utilization of varying degrees of light by sun and shade plants have become so well known that they have found their way into recent textbooks, even in America. His *Klima und Boden in ihrer Wirkung auf das Pflanzenleben* has a wide circulation and has run through two editions.

The appearance of an English translation of the second edition of this book[5]

[5] ASHBY, ERIC, Environment and plant development. Transl. from "Klima und Boden in ihrer Wirkung auf das Pflanzenleben" by Dr. HENRIK LUNDEGÅRDH. pp. ix+330. *Figs. 87.* Edward Arnold & Co. London. 1931.

will serve to make his results familiar to a larger audience, and will encourage further research in similar fields. The translator seems to have done his work well and shows that he is familiar with the problems of experimental ecology. The English is clear cut and as simple as the subject will permit.

The scope of the volume may be gathered from the chapter headings: light factor; temperature factor; water factor; ecological properties of the soil; physical structure and aeration of the soil; chemical properties of the soil; soil micro-organisms; the carbon dioxide factor; and principles of experimental ecology. In every case the discussion abounds in the results of experimental research, and often the results are given in the form of tables and graphs. Many of these are original; others are taken from the results of other recent investigators. Perhaps the best portions of the work are in the discussions of light, certain phases of temperature, the interpretation of xerophytism, and carbon dioxide as an ecological factor.

The bibliography is rather extensive, but would have been much improved had the titles of the articles cited been given. There is both an author and a subject index. The latter seems carefully made and serviceable, although rather brief. Altogether the book is one that no ecologist can afford to be without, and the translator has rendered good service to ecology by making it available to a wider circle of readers.—G. D. FULLER.

Alpine plants in gardens

The mountain climber who wishes to bring a portion of the alpine landscape home with him, the botanist interested in the problems of mountain vegetation, and the landscape gardener, will all find interest and instruction in this recent book on alpine plants in the garden.[6] While written from the viewpoint of the landscape gardener, it contains much accurate information for the botanist.

The first of the four parts into which the book is divided deals with the preparation of the rock garden and contains chapters on building the garden, the preparation and watering of the soil, and the various methods of obtaining and propagating alpine plants. There follow a series of sketches of the mountains where the flowers grow, the descriptions being largely confined to the mountain systems west of the Rockies. Photographs of mountain meadows and of alpine gardens are taken from the same western region. The third and fourth parts deal with the plants themselves. A long alphabetically arranged list contains many of our most beautiful alpines. The descriptions contain the scientific names and accurate information regarding the size, appearance, and habitat of each plant. To the botanist this carefully annotated list constitutes the most valuable portion of the book. There follow lists of species grouped according to habit, growth forms, and cultural suitability. These lists and the entire organization of the volume tend to make the information contained within it easy of reference.—G. D. FULLER.

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[6] MCCULLY, A., American alpines in the garden. pp. 251. *Pls.* 17. Macmillan Co. New York. 1931.

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Bacterial plant pathogens

Among recent additions to the literature welcome to the plant pathologist and bacteriologist, the volume by ELLIOTT[7] will prove especially useful. A summary compilation of the data pertinent to bacterial plant pathogens has been a crying need for many years. Something was needed in the English language to supplement SMITH'S *Bacteria in relation to plant diseases* and his *An introduction to bacterial diseases of plants*, and BERGEY'S *Manual of determinate bacteriology*. SMITH'S volumes were not designed to give a complete record of bacterial plant diseases, while BERGEY'S manual by intent is merely a key to bacterial plant pathogens.

The volume contains an alphabetical list of the bacterial plant pathogens and of a few possible pathogens. together with a list of bacteria usually associated with these. Under the heading of each organism are given its description, its synonymy, the symptoms incited by it, its hosts, its geographical distribution, and the literature. The literature is remarkably complete and this feature alone is sufficient to commend the volume to pathologists and bacteriologists. The names adopted in this volume are according to the system of SMITH'S modification of MIGULA'S system. Time only can tell whether this was a wise choice.—G. K. K. LINK.

Botanical explorations

An interesting little book has just appeared, written by PALMER,[8] a Fellow of the Royal Geographical Society, with an introduction by the late J. ARTHUR HARRIS. This volume contains a modest account of mountain climbing and botanical exploration in the Selkirk, Cariboo, and Rocky Mountains of western Canada. HOLWAY began mountain climbing while on the botanical staff of the University of Minneosta at the age of nearly fifty, and during the remaining sixteen years of his life he climbed scores of the highest peaks of western Canada. Many of these were first ascents and in reaching them he traversed much unexplored territory. He is entitled to rank among the foremost mountaineers of America, and this volume seems a fitting memorial to a daring, original, and modest explorer.—G. D. FULLER.

Sylloge Fungorum

Volume XXV of SACCARDO'S *Sylloge Fungorum, Supplementum Universale*, has made its appearance.[9] It is devoted to Myxomycetae, Myxobacteriaceae, Deuteromycetae, and Mycelia sterilia.—G. K. K. LINK.

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[7] ELLIOTT, CHARLOTTE, Manual of bacterial plant pathogens. pp. vii+349. Williams and Wilkins Co. Baltimore. 1930.

[8] PALMER, H., Edward W. D. Holway: pioneer of the Canadian Alps. pp. xiii+81. University of Minnesota Press. Minneapolis. 1931.

[9] Sylloge Fungorum, Vol. XXV. Supplementum universale, Pt. X. Edit. ALEX. TROTTER. 8vo. pp. 1093. Pergola, Italy. 1931.